GENETIC DISSECTION AND MOLECULAR APPROACHES OF ROOT MORPHOLOGY OF CANOLA

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Muhammad Arif Uz Zaman

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By

MUHAMMAD ARIF UZ ZAMAN

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Mukhlesur Rahman
Chair

Dr. Phillip E. McClean

Dr. David P. Horvath

Dr. Luis del Rio-Mendoza

Approved:

April 2, 2014
Date

Dr. Richard Horsley
Department Chair

ABSTRACT

The inheritance of root vigor and days to flowering were studied in the F_2 populations derived from winter- and spring-type crosses and their reciprocal crosses. Root vigor was found to be controlled by three genes whereas, a single dominant gene was found to be responsible for days to flowering. Positive and significant correlation was found between seed yield and yield attributing traits, seed yield and root length, days to flowering and root length. Days to flowering was negatively correlated to seed yield. Linkage map was constructed with 262 SNP and 3 SSR markers. Two QTL for both root vigor and days to flowering were identified. One of the root vigor QTL was assigned on *B. rapa* chromosome A03 and *B. oleracea* C09, and two putative candidate genes, *ASA1* and *RLF* were identified. Flowering time QTL was assigned on the A07 and C01, and a candidate gene, *ATH1* was detected.

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CHAPTER 1. GENERAL INTRODUCTION

Brassica napus L. (AACC, 2n= 38) is one of the youngest cultivated species. It is believed to be originated from the natural interspecific hybridization of another two diploid *Brassica* species, *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18) in Mediterranean region of Eastern Europe. Natural *Brassica* oilseed species contain high erucic acid in the oil and high glucosinolates content in the meal. High erucic acid consumption is a risk for human health and high glucosinolates content in the meal adversely affect the taste of the meal. Canola was developed by Canadian scientist Dr. Baldur Stefansson in 1974 by lowering the glucosinolates and erucic acid content in *B. napus.* The word Canola stands for "Canadian Oil Low Acid". Now-a-days, canola includes *B. rapa* and *B. napus* cultivars, which have low erucic acid content (less than 2%) in oil and low glucosinolate content (less than 30 micromoles/gram) in meal. Canola oil is now a well-established important oilseed crop throughout the world. It is also a great source of animal feed and has been used for biodiesel production. North Dakota is pioneering the canola production in United States, holding 81 % of the total country acreage (NASS, USDA, 2013).

There are three types of canola habitats, winter, semi-winter and spring. Winter type of canola exhibit vigorous growth with strong stem, big leaves, dense root system and needs vernalization for flowering. On the other hand, spring type is a comparatively less vigorous plant with lighter root system and no need of vernalization for flowering. Besides these morphological characteristics, yield is the most important criteria where these two habitats differ greatly. Winter canola gives higher production than spring canola. Winter canola is mainly grown in moderate

temperature climate like that of Western Europe and spring canola is mainly grown in Canada, the United States, Australia, India and Bangladesh. The semi-winter types are intermediate of winter and spring habitats. They do not have frost hardiness like winter canola, therefore, they are cultivated in moderate winter temperature region like Central China (Wang et al., 2011).

Unfortunately, canola growers have to depend on comparatively low yielding spring canola due to the severe winter hardiness in the growing areas of Canada and the United States, including North Dakota. Most breeding programs in this region are mainly aimed to improve yield and seed oil content of spring canola. The allelic variation and genetic diversity is reduced to a great extent in the spring canola varieties developed in the last century in Canada and Australia due to focusing too much on spring canola improvement (Fu and Gugel, 2010, Cowling, 2007). According to different diversity studies in canola, it is now well established that spring, winter and semi-winter germplasms belong to completely different genetic groups with higher genetic diversity between them (Rahman, 2013). Therefore, winter germplasms with higher seed yield and superior agronomic characteristics could serve as a vital source of genetic variation to improve spring canola attributes.

Strong and dense root system facilitates higher water uptake and nutrient acquisition from the soil resulting good crop stand, even in the adverse situations. Furthermore, a strong root system is a prerequisite for optimal plant growth, which ultimately boost up the yield (Marschner, 1998). In canola, there are few studies describing that different nutrient acquisition capability varies with differential root traits. Solaiman et al. (2007) reported a positive correlation between shoot phosphorus (P) content in canola and root length under low availability of P in soil and also found a strong positive relationship between the P removed from the soil and root length in canola (*B. napus* L.). Increased lateral root length and rate of biomass

accumulation have been positively correlated with phosphorus uptake and yield in canola (Duan et al., 2009). However, none of these studies directly correlates root traits and ultimate yield. Rahman and McClean (2012) first identified that three genes are responsible for root vigor in canola and showed significant positive correlation of root traits with yield and different yield contributing traits.

Molecular approaches in addition to the classical breeding techniques provide a cost and time effective screening tool for selection of desirable traits (Hawkins et al., 2005). Information gained from QTL analysis can be used successfully as a tool to improve traits which are difficult to measure or have low heritability and could serve as the starting point for marker-assisted selection (Collard et al., 2005). Identification of quantitative trait loci (QTL) for root traits in canola can provide us with useful indications on the genetic basis of root traits in canola. This is the first approach to identify QTL associated with root traits in canola. However, QTL responsible for flowering time in canola as well as in other *Brassica* sp. have been identified by many scientists, i.e. Ferreira et al. (1995), Osborn et al. (1997), Delourme et al. (2006), Zhao et al. (2010) and Camargo et al. (1996).

The major objectives of this study were:

1. To study the inheritance of root vigor and days to flowering.
2. To study the correlation of different root traits with yield and different yield attributing characteristics.
3. To identify the QTL associated to root morphology and flowering time.

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CHAPTER 2. LITERATURE REVIEW

2.1. Rapeseed/*Brassica napus* L.

2.1.1. History of origin

Brassica napus L. is a relatively young species, also known commonly as rapeseed, swede rape, Argentine rape, oil rape or oilseed rape. It has relatively short evolutionary history and no true wild form have been found (Go´ mez-Campo and Prakash, 1999). It is believed to have originated from spontaneous interspecific hybridization between *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18) genotypes (U, 1935) in Mediterranean region of south-western Europe (Cruz et al., 2007). The proof of its use in India has been recorded as early as 2000 BC (Colton and Potter, 1999). It has been cultivated in Europe since13th century, mainly to produce lamp oil (Colton and Sykes, 1992).

2.1.2. Taxonomy and morphology

The species *Brassica napus* L. belongs to the genus *Brassica* in the Brassicaceae family. This family consists of approximately 375 genera and 3200 species. The genus, *Brassica*, consists of approximately 100 species (Thomas, 2003). *B. napus* flowers are bisexual, and formed in branching type of inflorescence. The flowers contain four petals, four sepals, six stamens and a pistil of two carpels. The ovary is superior type, positioned above receptacle (Baily, 1976). *B. napus* is a self-pollinating crop. Each and every flowers produce large amount of pollen, which out-compete the pollens from adjacent flowers to fertilize. However, 12-47% cross-pollination can occur due to insect pollinators, wind or physical contact (Becker et al., 1992; Williams et al., 1986). Seeds are formed in a capsulated two-celled fruiting body, which is commonly called a pod or silique (Baily, 1976).

2.1.3. *Brassica* U triangle

Three allotetraploid and three diploid species of the genus *Brassica* are genetically linked. This theory, first described by Nagaharu U (1935), is commonly known as 'Triangle of U' **(**Figure 2.1**)**. According to this theory, three diploid Brassica species, *Brassica oleracea* (CC, 2n=18), *Brassica nigra* (BB, 2n=16) and *Brassica rapa* (AA, 2n=20) hybridized in three independent events to produce three allotetraploids, *Brassica juncea* (AABB, 2n=36), *Brassica napus* (AACC, 2n=38), and *Brassica carinata* (BBCC, 2n=34). This theory has had various important implications in Brassica breeding; specially, in trait improvements (i.e. yellow seed coat color, low glucosinolate level) through interspecific crosses, based on this interspecific relationship (Branca and Cartea, 2011).

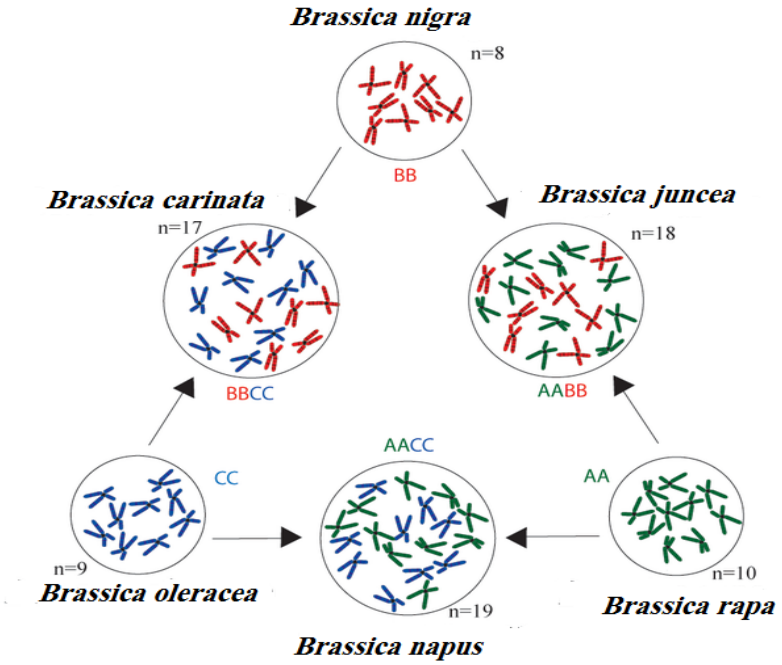

Figure 2.1. Triangle of U showing the genetic relationship among six *Brassica* sp. [source: Triangle of U in Wikipedia "http://en.wikipedia.org/wiki/File:Triangle_of_U_Simple1.PNG"]

B. napus (AACC, 2n=38) contains two sets of genome (A and C genome), as it originated through the hybridization of two diploid species, *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18) (U, 1935). *B. napus* was developed artificially later from *B. rapa* and *B. oleracea* by Olsson (1960), which supports the theory of U (1935).

2.2. Canola

2.2.1. Rapeseed and canola

Two species, *B. rapa* and *B. napus*, are commonly known as rapeseed. The "double low" rapeseed cultivars, which have low erucic acid content (less than 2%) in oil and low glucosinolate content (less than 30 micromoles/gram) in meal, are known as 'Canola' (Shahidi, 1990). The name 'Canola', stands for "Canadian Oil Low Acid", is a trademark of Canadian Canola Association.

2.2.2. History of canola

The ancient use of rapeseed was limited to lamp-fuel. However, after the invention of motorized vehicles and ships in the early years of the last century, people started to use rapeseed oil as lubricant. Rapeseed oil performed well as lubricant, as it has high erucic acid content in the oil. Canada started to grow higher amount of rapeseed during the World War II years due to increasing demand of lubricants for war and merchant ships. The first registered high erucic acid and high glucosinolate rapeseed cultivar 'Golden' was released in 1954 in Canada (Stefansson, 1983). Rapeseed oil was not well accepted as edible purposes due to its low quality and high erucic acid content. The high glucosinolates content in the rapeseed meal also reduced its acceptability for use in using animal and poultry feed. However, in 1963, first and only naturally occurring low erucic acid *B. rapa* line 'Liho' was discovered at University of Manitoba

(Stefansson and Hougen, 1963). This event opened the door for developing rapeseed line with low erucic acid. First commercial low erucic acid *B. rapa* cultivar 'Oro' was released in 1966. Subsequently, a low glucosinolate content *B. napus* line 'Bronowski' was identified in 1967 in Poland. Therefore, it triggered the opportunity to develop the 'double low' (low in both erucic acid and glucosinolates content) rapeseed lines. After years of research, finally a double low *B. napus* line, 'Tower', was developed by Dr. Baldur Stefansson in 1974 at University of Manitoba (Brown et al., 2008).

2.2.3. Health benefits of canola

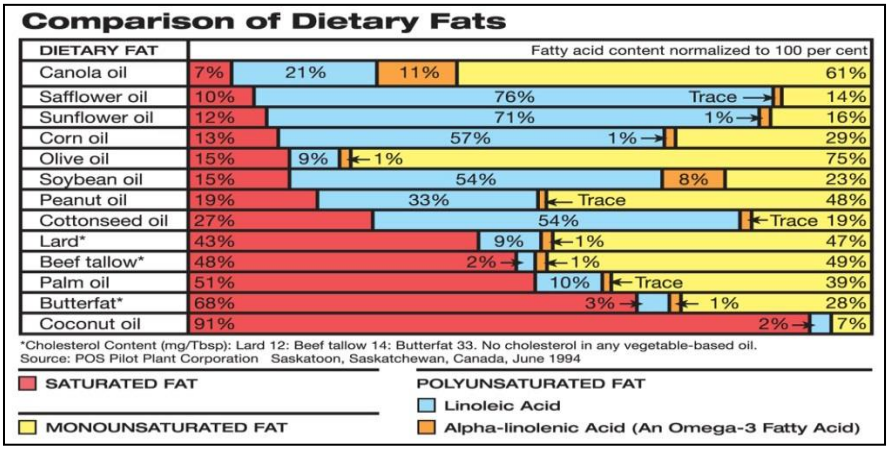

Figure 2.2. Comparison of dietary fats in different types of oil. (Source: POS Pilot Plant Corporation Saskatoon, Saskatchewan, Canada, June, 1994)

Rapeseed oil with high erucic acid is harmful for human health. Consumption of rapeseed oil with high erucic acid level may be involved in increasing the cholesterol level in blood and causeing fibrotic myocardium (Gopalan et al., 1974). Development of canola (double low rapeseed) overcomes these health issues regarding erucic acid. Due to low erucic acid level in canola oil, it becomes very healthy and digestible as well (Beare et al., 1963). Canola oil contains very low saturated fatty acid and a very high monounsaturated fatty acid. Saturated fatty acid is

related to coronary heart diseases, while the monounsaturated fatty acid is heart-healthy and controls the blood glucose by lowering the bad cholesterol level (LDL). In addition, canola oil contains well balanced polyunsaturated fatty acids, linoleic acid and alpha-linolenic acid, in a ratio of 2:1 (Fig 2.2), which regarded as nutritionally favorable. Canola oil is also a good source of vitamin E (Canola Council of Canada, 2013).

2.2.4. Canola production

Currently, rapeseed/canola occupies the second position after soybean in terms of worldwide oilseed production, contributing 13.83% of the total production (Table 2.1). In North America, Canada is the prime producer of canola. However, canola acreage has increased significantly in the United States in recent years due to increasing demand of canola oil. In 1995, total US canola acreage was only 367,000 acres, while in 2013, total canola acreage is 1307000. In the United States, North Dakota alone holds more than 81 % of the total acreage (NASS, USDA, 2013).

Table 2.1. Production of major oilseed crops worldwide.

Commodity name	2009/10	2010/11	2011/12	2012/13	Jan 2013/14	Feb 2013/14
	----------Million MT----------					
Copra	5.71	44.3	47.78	46.07	44.51	44.08
Cottonseed	39.51	12.91	13.79	14.85	15.47	15.48
Palm Kernel	12.43	39.52	37.87	39.93	39.47	39.47
Peanut	35.92	60.58	61.48	63.02	70.07	70.12
Rapeseed	61.06	263.9	239.16	268.27	286.83	287.69
Soybean	260.4	33.63	40.64	36.4	43.68	43.3
Sunflower seed	32.14	5.88	5.57	5.79	5.82	5.82

Source: FAS, USDA, 2014

2.3. Roots

2.3.1. Background of root study

Among other constraints of crop production, edaphic stresses are the principal constraints (Cakmak, 2002). Scientific approaches to overcome these stresses and for crop yield gains are being used mainly from the last century after the sudden increase of the population throughout the world. High inputs of different fertilizers were the main approaches throughout the last century to obtain greater yield. Even very limited breeding programs were designed on the basis of nutrient uptake efficiency of crops. This resulted many high yielding varieties with very low nutrient uptake efficiency. To obtain future gains in crop production, main emphasis should be given to the enhancement of crop adaptation to different edaphic stress conditions (Lynch, 2007), This can be achieved by the genetic improvement of root apparatus traits associated with soil exploitation and nutrient acquisition (de Dorlodot et al., 2007).

2.3.2. Crop roots in relation to yield

A strong and dense root system is a prime criterion for good crop stand as it facilitates higher water uptake and nutrient acquisition from the soil, even in adverse situations. Pavlychenko (1937) first described the importance of plant roots to capture soil moisture and nutrients and maintain crop productivity. In addition, development of adequate root system plays a vital role for optimal plant growth; and optimal plant growth can significantly boost up the yield (Marschner, 1998).

A considerable amount of research in many crops were done to correlate yield with different root parameters such as, root length, root weight, root diameter etc. Strong relationship between root length and yield was illustrated in corn (MacKay and Barber, 1986) and in soybean (Brown and Scott, 1984). Ehdaie et al. (2010) found significant positive correlation of plant N

content; P and K uptake and grain yield with root biomass in wheat and suggested to develop wheat genotypes with superior root characteristics in breeding programs for higher grain yield. Drought-resistant maize genotypes with larger root systems are able to lift more water than drought sensitive genotypes with smaller root systems (Wan et al., 2000). Increasing evidence suggest that nutrient uptake efficiency might depend on the plant's ability to modify its root apparatus in order to increase the absorptive surface and the amount of soil volume explored (Doussan et al., 2003). A positive correlation was found between deep root system and seed yield in sorghum under drought condition (Jordan et al., 1983).

2.3.3. Inheritance of root system in canola

Unfortunately, no research was conducted to determine the inheritance of root system in canola until 2012. Rahman and McClean (2012) first studied the root system in a segregating F_2 population, derived from winter-spring crosses to determine the inheritance of the root system in canola. They reported that the winter type root system is dominant over the spring type and a trigenic effect is responsible for root system in canola.

2.3.4. Correlation between root traits and yield in canola

A few studies have focused on the relationship between root traits and seed yield in canola. Instead, most studies have focused on how root traits affect and direct moisture and nutrient uptake efficiency or how they facilitate good anchorage. Solaiman et al. (2007) reported a positive correlation between shoot P content in canola and root length under low availability of P in soil and suggested that the microbial community composition in rhizosphere may be a very important factor for the better growth of canola compared to wheat genotypes. They also found a strong positive relationship between the P removed from the soil and root length in canola. Increased lateral root length and rate of biomass accumulation has been positively correlated

with phosphorus uptake and yield in canola (Duan et al., 2009). None of these experiments indicated any relation of the differential nutrient acquisition ability caused by root trait differences to the seed yield. However, these factors can lead to increased seed yield indirectly.

Rahman and McClean (2012) first showed significant and positive correlations between different root traits and agronomic characters of canola, such as, days to flowering vs. root length, days to flowering vs. dry root weight, pods per plant vs. root length, root length vs. dry stem weight, root length vs. dry root weight, root length vs. seed yield, dry stem weight vs. dry root weight. Akhtar et al. (2008) reported that increased root biomass can lead to increase above ground biomass in *B. napus*. As strong root biomass have positive effect on above ground biomass and above ground agronomic traits are correlated to final seed yield, it can be hypothesized that root biomass is positively linked to final seed yield in canola. Koscielny and Gulden (2012) found that root length is the best indicator of seed yield at the one-to two-leaf and the three-to four-leaf stages of development in the field and growth room condition. This evidence demonstrates the potential of early root development in canola as a screening trait in breeding programs, for the selection of increased seed yield.

2.4. Flowering time

2.4.1. Flowering time in canola

Transition from vegetative to reproductive stage in flowering plants is very sensitive to climatic conditions (Koornneef et al., 2004). To ensure high productivity in crop plants, it is important to control timing of this transition (Zhao et al., 2010). Sometimes, this transition time needs to be controlled to avoid flowering under unfavorable climatic condition in a specific region (Andersen et al., 2005). Modification of flowering in Brassica crops is important to

expand the geographical range of cultivation (Lagercrantz et al., 1996). For example, early flowering is an important trait of interest in breeding programs in Canada, because it can facilitate cultivation of *B. napus* further north (Murphy and Scarth, 1994).

In canola, there are three different habitats based on flowering trait. The spring types are early flowering and they do not need vernalization for flowering. They are mainly grown in Canada, the Unites States, India and Australia. In contrast, winter types need vernalization for flowering and are grown in moderate temperature climates, such as, Western Europe. The semi-winter types are sown before winter and they flowered after winter. They do not have frost hardiness, therefore, cultivated in moderate winter temperature region like Central China. (Wang et al., 2011)

2.4.2. Flowering time genetics

Flowering time is a very complex genetic trait and believed to be controlled by several major and minor genes in different plants. Thurling and Das (1979) first mentioned that flowering time in *B. napus* is controlled by two types of gene; one type is responsible for differential vernalization responses and the other for earlier flowering in plants which do not need any vernalization. They also reported that mainly two genes were responsible for different vernalization responses in *B. napus* and one of them had significantly greater effect than the other. Light et al. (2005) evaluated winter-spring segregating F_2 population and found that one major gene is responsible for vernalization requirement for flowering in *B. napus*. Ferreira et al. (1995) identified three major QTL, one of which is describing most of the flowering time variation while other two have only minor effects. Lagercratnz et al. (1996) concluded that one major gene affect the flowering time variation in *B. nigra* while another minor gene effect might be present in addition. Rahman and McClean (2012) also concluded that one major gene, along

with one or more minor genes may be responsible for flowering time in populations derived from winter and spring type crosses.

2.6. Molecular approaches

2.6.1. Background of molecular study

The prime goal of every breeding program is to increase yield by improving agronomic traits through effective selection. However, few of the agronomic traits are qualitative in nature; rather, they are mostly quantitative in nature. Quantitative traits are controlled by multiple genes of varying effect. Furthermore, they interact with the environment and show continuous variation in phenotypic expression. These facts made difficult to improve them through conventional breeding techniques. Here lies the importance of molecular approach in plant breeding. Molecular approaches in addition to the classical breeding techniques provide a cost and time effective screening tool for selection of desirable traits (Hawkins et al., 2005). Development of high density marker techniques made it possible to detect quantitative trait loci linked to the markers. Quantitative trait loci, or QTL, are areas of the genome that include genes or other sequences that affect quantitative traits. These molecular techniques can be used as effective tools in the selection and screening process for complex traits, which can significantly reduce the breeding cycle and sometimes cost too. Though the basic idea of molecular approaches in plant breeding is to identify a marker that linked to a region or gene of interest, it also includes mapping of the genes, marker development and transfer the identified genes to plants which lead to a new term, Marker Assisted Breeding (MAS).

2.6.2. Molecular markers

The necessity of genetic markers for dissecting the complex traits into individual QTL, has long been described (Sax, 1923; Rasmusson, 1933; Thoday, 1961; Tanksley et al., 1982).

Originally, morphological markers were used before molecular markers to dissect quantitative traits (Sax, 1923; Rasmusson, 1933). In 1950s, protein or biochemical markers became popular (Hunter and Markert, 1957). Two types of protein markers were used, isozimes and storage proteins, separated mainly by SDS-PAGE (Sodium Dodecylsulfate Polyacrylamide Gel Electrophoresis).

Since, it was difficult to construct high density genetic maps with these markers only, scientists were in search for more efficient marker technologies. Restriction Fragment Length Polymorphism (RFLP) is the first genetic markers based on the concept of variations at DNA level. RFLP markers were first used in viruses (Grodzicker et al., 1974) followed by human globulin gene cluster (Jeffreys, 1979). In this technique, DNA are extracted from different individuals, digested with the help of restriction enzymes and the fragments containing specific sequences are scored based on size difference. Since then many other molecular marker techniques have been developed and used for genetic map construction and gene tagging. Generally, DNA markers can be classified into two types: hybridization-based markers, such as, Restriction Fragment Length Polymorphisms (RFLP) (Weber and Helentjaris, 1989), Fluorescence In-situ Hybridization (FISH) (Pinkel et al., 1986), and PCR-based markers, such as, Amplified Fragment Length Plymorphism (AFLP) (Vos et al., 1995), Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990), Simple Sequence Repeats (SSR) (Herne et al., 1992), Sequence Tagged Sites (STS) (Fukuoka et al., 1994), Single Nucleotide Polymorphism (SNP) (Gabor et al., 1999), Sequence Related Amplified Polymorphism (SRAP) (Li and Quiros, 2001) and others.

2.7. Quantitative trait loci mapping

QTL mapping is a statistical approach, in which, both genotypic and phenotypic data are linked together and analyzed to explain the genetic basis of variation in quantitative traits (Falconer and Mackay, 1996; Kearsey, 1998; Lynch and Walsh, 1998). QTL mapping methods can be classified into two groups, single marker analysis and multiple markers analysis or genome wide scan. In multiple marker analysis or genome wide scan methods, it is possible to take into account several markers but statistically complex as numerous comparisons are performed during the analysis. Hence, it reveals more information comparing single marker analysis. Genome wide scan methods include standard interval mapping, composite interval mapping, and multiple imputation method.

2.7.1. Single marker analysis

Single marker analysis was used mostly in the early years of QTL mapping era. The goal was to detect the markers that linked with a QTL. In this method, one way analysis of variances is performed for each marker. Though this method effectively identifies QTL linked to a marker, it does not give any precise information about the location of the QTL. Moreover, it is not possible to detect the magnitude of the effect of the identified QTL through this method. Nevertheless, researchers sometimes prefer this method if any prior knowledge on a loci is available (Rafalski, 2010).

2.7.2. Interval mapping

Standard interval mapping (IM) method facilitates the researchers to identify the location of a QTL and its degree of effect. This method was first proposed by Lander and Botstein (1989). In this method, the likelihood of existence of a QTL between the interval areas of each adjacent pair of markers along the chromosome is evaluated. The log value of odds ratio of

likelihood or LOD value is calculated for one vs. no QTL present at a particular point (Lander and Botstein 1989). An alternative approach using multiple regression was also developed to increase the accuracy and simplicity (Hayley and Knott, 1992; Martinez and Curnow, 1992). Software package Mapmaker/QTL (Lincoln et al., 1992) is widely used in interval mapping method. However, there are still some limitations of this method. In closely linked QTL, a ghost QTL might appear to be hiding the original two QTL (Moreno, 1992). Furthermore, biased estimation of a QTL may occur as this method deals with the effects of additional QTL as sampling variation.

2.7.3. Composite interval mapping

Composite Interval Mapping (CIM), a modification of IM method, was proposed to solve the problem associated with 'ghost QTL' (Zeng 1993, 1994; Jansen, 1993b). This method combines the regression and maximum likelihood procedure. The analysis in this method is similar to that of IM but additionally it takes into account the variation from other QTL by including cofactors in other regions of the genome. CIM method has higher resolution and detection power than IM (Zeng, 1994).

2.7.4. Multiple imputation method

The multiple imputation method (MI) is considered as the most useful approach to explore multiple QTL models (Sen and Churchill, 2001; Broman and Sen, 2009). It deals with the missing genotypic data in a different manner comparing other methods. The procedure involves predicting the unknown genotypes between markers based on the known genotypes at the two flanking markers. These predictions are based on the probabilities of the recombination events between the markers. After the data are imputed or predicted, t-tests are performed at each

position (using each imputation). Finally, the results are averaged from each t-test and expressed as a LOD score (Broman and Sen, 2009).

2.7.5. QTL studies for root traits in different crops

The collection and measurement of root traits are highly laborious. Furthermore, the plastic nature of root growth in most of the crops makes the measurement and accountability even more difficult. Root plasticity refers to the preferential growth of the root to the area of high moisture and nutrient. Thus, root growth differs from plant to plant, even if a small change in soil environment. These facts increase the difficulties in taking decisions about root genetic analysis for further selection in breeding program. However, identification of quantitative trait loci accounted for root traits variation, by using modern QTL mapping approach, can provide useful indication to understand the genetic basis of root traits. Furthermore, development of molecular markers linked with QTL associated with root traits and grain yield can facilitate the marker assisted selection to improve productivity.

Unfortunately, no research has been done previously to identify QTL for root traits in Brassica sp. However, there are few reports stating QTL analysis for root traits in other crops. Tuberosa et al. (2002) performed QTL mapping for rice root traits with the help of total 176 markers (RFLP, AFLP and SSR). They detected 11, 7 and 9 QTL for primary root length, primary root diameter, and primary root weight, respectively. Six QTL were identified by Yadav et al. (1997) for both total root weight and maximum root length in a double haploid population of rice. These QTL accounted for 43% and 49% of the phenotypic variation for total root weight and maximum root length, respectively.

In maize, Lebreton et al. (1995) identified the QTL regions, which influencing root pulling resistance at flowering and other morphological and physiological traits in an F_2

population grown under greenhouse conditions. Guingo et al. (1998) described the co-location of QTL for root architecture and above-ground biomass production in recombinant inbred line population. QTL analysis for seminal root traits in maize was performed by Zhu et al. (2006). In this analysis, they identified one main-effect QTL (explaining 11% variation) associated with seminal root length and three QTL (explaining 25.4% variation) associated with seminal root number under low phosphorus condition. In addition, they detected two QTL for seminal root length explaining total 22.8% of the phenotypic variation and three QTL for seminal root number explaining total 24.1% of the phenotypic variation under high phosphorus condition.

2.7.6. QTL studies for flowering time in *Brassica* sp.

Many quantitative trait loci for flowering time (both large and small effect) have been identified so far in different Brassica sp. Ferreira et al. (1995) identified three major QTL in *B. napus*, one of which is describing most of the flowering time variation while other two have the minor effects only. Osborn et al. (1997) identified one largest-effect QTL (explaining 50% of variation), which related to a major vernalization-responsive flowering time QTL (*VFN2*) in *B. napus*. Delourme et al. (2006) performed QTL analysis for earliness of flowering in two double haploid populations derived from *B. napus* and detected three and nine QTL respectively. Quijada et al. (2006) identified ten QTL for days to flower. A major QTL corresponding with *BrFLC2* as the candidate gene on linkage group A02 was identified by Lou et al. (2007) in several *B. rapa* populations evaluated under different environmental conditions. Zhao et al. (2010) identified three flowering time QTL and two vernalization responsive QTL in two linkage groups. In *B. napus,* co-localization of flowering time QTL with yield and yield-related QTL has also been reported in other studies (Udall et al., 2006; Basunanda et al., 2010; Chen et al., 2010). Two QTL associated with both flowering time and flowering-time index and one additional QTL

for flowering-time index only was identified by Camargo et al. (1996) in *B. oleracea.* Rahman et al. (2014) identified a single QTL for days to flowering in *B. rapa* that explains 21.4% phenotypic variation.

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CHAPTER 3. INHERITANCE AND CORRELATION STUDY ON DIFFERENT AGRONOMIC AND ROOT CHARACTERISTICS IN CANOLA (*Brassica napus* L.*)*.

3.1. Abstract

Winter and spring types canola (*Brassica napus* L.) exhibit completely opposite phenomena in terms of root characteristics and flowering time. Vernalization required winter types have stronger and more vigorous root system comparing to that of non-vernalized spring types. Inheritance of root vigor and days to flowering were investigated in segregating populations derived from crosses and reciprocal crosses between two winter types and two spring types parents. A total of 717 F_2 individuals from all cross combinations were evaluated for root vigor and a trigenic effect was detected controlling this trait. A total of 653 F_2 plants were analyzed to study the days to flowering. A single dominant gene was found to be responsible for the differences in days to flowering between these cultivars. In addition, a correlation study among yield, different yield attributing characteristics and root parameters was performed. Seed yield was positively correlated with number of pods per plant, pod length, seeds per pod and root length in both spring vs. winter and winter vs. spring crosses. Root vigor and root length were also positively correlated with days to flowering. Strong negative correlation was detected between days to flowering and seed yield in both types of crosses.

Key Words: inheritance, correlation, root traits, days to flowering

3.2. Introduction

Canola (*B. napus* L.) was developed to overcome limiting attributes, i.e. high erucic acid in oil and high glucosinolates content in the meal, of the traditional oilseed rape species. In addition, canola has some other tremendous attributes such as, high seed oil percentage (< 40%), very low saturated fatty acid percentage and a good ratio of polyunsaturated fatty acids in oil, which makes it healthier for human consumption. Although Canada is the prime producer of canola in North America, the United States has significantly increased acreage in recent years. The state of North Dakota alone holds the 84 % (avg.) of total canola acreages in United States in last seven years (NASS, USDA, 2013). It is now well established rotation crop with wheat in ND.

There are three types of canola habitats, winter, semi winter and spring canola. According to different diversity studies in canola, it is now well established that spring, winter and semi-winter germplasms are in completely different genetic groups with higher genetic diversity between them (Rahman, 2013). Among them, winter and spring types shows different root architecture, winter types have vigorous and dense root system while spring types possess less vigorous light root system. Due to extreme winter hardiness, comparatively poor yielding spring canola is the strategic crop in North Dakota and Canada. Canola breeders highly concentrated to improve the spring canola only in Canada and Australia in the last century which resulted a reduced genetic diversity in the improved spring cultivars in these regions (Fu and Gugel, 2010; Cowling, 2007). Therefore, winter germplasms with higher yield and superior agronomic characteristics could serve as a vital source of genetic variation to improve spring canola attributes.

Strong and dense root system is a prime criterion for good crop stand, as it facilitates higher water uptake and nutrient acquisition from the soil, even in adverse situations. Pavlechenko (1937) first describe the importance of crop root to uptake water and nutrient. Besides water and nutrient uptake, crop root plays a vital role for optimal plant growth and good crop stand which ultimately leads to higher crop yield (Marschner, 1998). Until now, there are very few studies available to understand the genetic nature of the root architecture in canola. Rahman and McClean (2012) first described that root vigor is controlled by three genes in the populations derived from winter and spring type canola. Root and yield are positively correlated and there are various reports describing the correlation of different root characteristics with yield in different crops. Strong relationship between root length and yield was illustrated in corn (MacKay and Barber, 1986) and in soybean (Brown and Scott, 1984). Ehdaie et al. (2010) found a significant positive correlation between plant N content, P and K uptake and grain yield with root biomass in wheat and suggested to develop wheat genotypes with superior root characteristics in breeding programs for higher grain yield. A positive correlation between root length and seed yield was shown by Rahman and McClean (2012).

Flowering is the transition of flowering plants from the vegetative to reproductive stage and very sensitive to different environment (Koornneef et al., 2004). Controlling or modifying of this transition time is very important in breeders' point of view to ensure high crop productivity (Zhao et al.; 2010) by avoiding unfavorable climatic conditions in specific regions (Andersen et al.; 2005) or by expanding the geographical range of cultivation (Lagercrantz et al., 1996). Flowering time in winter and spring canola is also distinct from each other. Spring type canola does not need vernalization for flowering whereas, the winter type needs vernalization. To understand the genetics behind the variation of the flowering time, we need to detect the genes

that control this trait and which caused natural variation in flowering time (Ehrenreich et al., 2009).

Thurling and Das (1979) first mentioned that flowering time in *B. napus* is controlled by two types of gene; one type is responsible for differential vernalization responses and the other type is for earlier flowering in plants which do not need any vernalization. They also reported that mainly two genes are responsible for different vernalization responses in *B. napus* and one of them has significantly greater effect than the other. Light et al. (2005) evaluated winter-spring segregating F_2 population and found that one major gene is responsible for vernalization requirement for flowering in *B. napus*. Ferreira et al. (1995) identified three major QTL, one of which is describing most of the flowering time variation while other two have the minor effects only. Osborn et al. (1997) identified one largest-effect QTL (explaining 50% of variation), which related to a major vernalization-responsive flowering time QTL (*VFN2*) in *B. napus*. Lagercratnz et al. (1996) concluded that one major gene affect the flowering time variation in *B. nigra* while another minor gene effect might be present in addition. Rahman and McClean (2012) also concluded that one major gene, along with one or more minor genes may be responsible for flowering time in canola. Considering all these factors, our objectives are, (i) to study the inheritance of root morphology and days to flowering in canola and (ii) to determine the correlation of seed yield with different yield attributing characteristics and root parameters.

3.3. Materials and methods

3.3.1. Plant materials

The spring type canola cultivars ‘Regent’ (PI431572) and ‘Legend’ (PI601504) were obtained from USDA-ARS National Plant Germplasm System. The winter type varieties

'Lorenz' and 'Lagoda' were obtained from University of Alberta, Canada. All the parents were self-pollinated for four to five generations to develop pure breeding lines. The pure bred spring and winter type lines were crossed (F_1) and reciprocally crossed (F'_1) with each other. The F_1s from Lorenz × Legend, Lagoda × Regent and F'_1s from Legend × Lorenz, Regent × Lagoda were selfed further to produce the F_2 generation for segregation analysis. Backcrosses were performed in F_1s (Lorenz × Legend, Lagoda × Regent) by all the four parents. The F_1, F'_1, F_2 and BC_1 populations were grown in a greenhouse at 23±1°C (day and night). The plants in the greenhouse were provided with a 16-h photoperiod provided by natural sunlight supplemented with 400 W HPS PL 2000 lights (P.L. Light Systems Inc. ON, Canada). The seeds were sown in 6-inch (diameter) x 6-inch (depth) pots filled with Sunshine-Mix-1 (Sun Gro Horticulture, BC, Canada). Plants were watered daily and fertilized with water-soluble 20-20-20 fertilizer.

3.3.2. Vernalization required for winter-type parents

Four weeks old seedlings were transferred into a vernalization chamber at a temperature of 4±2˚C with a 12-h photoperiod provided by GE Ecolux F32T8 SP35 Eco (32 W T8) style bulbs (General Electric Company) and vernalized for 5 weeks. The seedlings were then transferred into a cold room at 12±3°C for 3 days and finally transferred to the original greenhouse conditions.

3.3.3. Days to flowering

Data on days to flowering were recorded on the basis of days required to the 1st flowering of each plant from the seeding day in the greenhouse.

3.3.4. Agronomic data

Data on plant height at maturity, number of branches per plant, number of pods per plant, pod length, number of seeds per pod were taken from each plant were recorded.

3.3.5. Root vigor data

Root vigor scoring was conducted at 50% of the flowering stage on a scale of 1-5 according to Rahman and McClean (2012), where, score-1 has weak bottom and surface roots, those observed in spring-type parents, score-2 has more bottom and surface roots, score-3 has intermediate bottom and surface roots, score-4 has strong bottom and surface roots, and score-5 has the strongest bottom and surface roots, those found in winter-type parents (Fig. 3.1). Roots were extracted after harvesting the pods by washing the root mass under running tap water. Special cares were taken during root collection. The extracted root dried in 60°C for 3 days and tap root length was recorded.

Figure 3.1. Root Vigor score on a scale of 1-5 (left to right) (1= weak bottom and surface roots, those observed in spring-type parents, 2 = more bottom and surface roots, 3 = intermediate bottom and surface roots, 4 = strong bottom and surface roots, and 5 = the strongest bottom and surface roots, those found in winter-type parents)

3.3.6. Root system inheritance

Inheritance of root traits was calculated on the basis of the data obtained from the F_2 and the backcross plants derived from the aforementioned crosses. The root vigor data was divided into two groups. The plants with root score-1 were in the first group, and all other root types (root score 2-5) formed the second group. Chi-square (χ^2) goodness of fit test was used to check expected versus observed phenotypic segregation ratios for F_2 and backcross data. The data were pooled for combined analysis. Before pooling the data for the four crosses, χ^2 homogeneity tests were conducted to determine whether the data could be pooled or not (Strickberger, 1985).

3.3.7. Correlation study

Data from root (i.e. root length and root vigor score) and yield attributing traits (i.e. plant height at maturity, number of branches per plant, number of pods per plant, number of seeds per pod, pod length, plant dry matter, days to flower, seed yield) were used for the correlation study. The Pearson correlation coefficient of these traits except root vigor was studied using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). The correlation between the root vigor and all other traits were determined by Point-biserial correlation analysis by using Microsoft Office Excel 2013 program.

3.4. Results

3.4.1. Root vigor inheritance

There were distinct differences in the root system of winter and spring parents. Vigorous root system was observed in all winter type parents, the F_1 and the F'_1 plants. In F_2, a total of 717 plants from two crosses (Lorenz × Legend, Lagoda × Regent) and their reciprocal crosses were evaluated. All plants were divided into two groups based on the root vigor, plants with weak root

and plant with vigorous root groups. All four F_2 population derived from aforementioned two crosses and their reciprocal crosses were fit with the trigenic segregation ratio 63:1 for strong root: weak root except Lorenz × Legend cross which does fit both 63:1 and 15:1 ratio (Table 3.1). However, the pooled data (χ^2 = 0.129, P= 0.72) was consistent with a Mendelian 63:1 phenotypic segregation ratio for pooled vigorous roots and weak roots. The test of homogeneity for four crosses on F_2 data (homogeneity χ^2 = 4.563, P = 0.21) indicated that the crosses could be pooled. These results also indicated that three gene loci are responsible for vigorous root system of *B. napus* in these crosses.

Table 3.1. Segregation of root vigor in the F_2 populations of winter type and spring type crosses

F_2 Population	Total Plants	Vigorous roots	Weak Roots	Segregation ratio					
				15:1			63:1		
				χ^2	df	*P*	χ^2	df	*P*
Lorenz × Legend	179	174	5	3.65	1	0.06	1.76	1	0.18
Legend × Lorenz	179	178	1	9.89	1	0.002	1.73	1	0.19
Lagoda × Regent	179	176	3	6.39	1	0.01	0.02	1	0.89
Regent × Lagoda	180	179	1	9.96	1	0.002	1.19	1	0.28
Total				29.89	4		4.70	4	
χ^2 (pooled data)	717	707	10	28.84	1	7.83E-08	0.13	1	0.72
Homogeneity χ^2 (total – polled)				1.05	3	0.79	4.57	3	0.21

3.4.2. Days to flowering inheritance

To determine the inheritance of flowering time we divided the whole population into two groups, plants that flowered and plants that did not flowered. Plants that were flowered show a continuous fashion of flowering in F_2 population. In both crosses and their reciprocal crosses, flowering started from 41 day and continued up to 78 days after planting. A total of 663 F_2 plants from all cross combinations were evaluated to check for the chi-square (χ^2) goodness fit test. The flowered and not flowered plants in both crosses and their reciprocal crosses were found to fit with 3:1 segregation ratio according to chi-square goodness fit test (Table 3.3). The pooled data

was also in accordance with the phenotypic ratio 3:1 for flowered: not flowered (χ^2=0.01, p=0.92). The test of homogeneity for all four crosses on backcross data (homogeneity χ^2 = 7.17, P = 0.07) indicated that the crosses could be pooled.

Table 3.2. Segregation of days to flowering in the F_2 populations of winter type and spring type crosses of canola

F_2 Population	Total Plants	Flowered	Not Flowered	Segregation ratio					
				3:1			15:1		
				χ^2	df	*P*	χ^2	df	*P*
Lorenz × Legend	142	103	39	0.46	1	0.50	106.77	1	5E-25
Legend × Lorenz	151	103	48	3.02	1	0.08	168.07	1	1.95E-38
Lagoda × Regent	180	140	40	0.74	1	0.39	78.37	1	8.54E-19
Regent × Lagoda	180	145	35	2.96	1	0.09	53.48	1	2.61E-13
Total				7.19	4		406.69	4	
χ^2 (pooled data)	653	491	162	0.01	1	0.92	383.84	1	1.82E-85
Homogeneity χ^2 (total – polled)				7.17	3	0.07	22.85	3	4.34E-05

3.4.3. Correlation study

A total of 170 F_2 plants derived from the crosses and reciprocal crosses of Lorenz × Legend and Lagoda × Regent were evaluated in the correlation study on different yield attributing characteristics (plant height, number of branch per plant, number of pods per plant, pod length, number of seeds per plant), root characteristics (root vigor, root length) and seed yield (Table 3.3). Significant positive correlation was found between plant height and number of branches (0.14, *P*=0.05; 0.30, *P*=0.001), plant height and pods per plant (0.22, *P*=0.001; 0.13, *P*=0.05), plant height and pod length (0.20, *P*=0.001; 0.20, *P*=0.001), number of branches/plant and pod length (0.13, *P*=0.05; 0.18, *P*=0.01), number of pods/plant and seed yield (0.38, *P*=0.001; 0.32, *P*=0.001), pod length and seeds/pod (0.64, *P*=0.001; 0.79, *P*=0.001), pod length and yield (0.16, *P*=0.01; 0.49, *P*=0.001), number of seeds/pod and seed yield (0.30, *P*=0.001; 0.51, *P*=0.001), days to flower and root length (0.32, *P*=0.001; 0.26, *P*=0.05), root length and

seed yield (0.22, *P*=0.001; 0.16, *P*=0.01) for both winter × spring and spring × winter types of crosses. In addition, in winter × spring crosses, days to flower and root vigor (0.23, *P*=0.05) and in spring × winter crosses, number of branches/plant and number of pods/plant (0.13, *P*=0.05) were positively correlated.

Significant negative correlations were found between plant height and days to flower in spring × winter cross only (-0.13, *P*=0.05), number of branches/plant and days to flower in spring × winter cross only (-0.13, *P*=0.05), pods/plant and days to flowering (-0.45, *P*=0.05; -0.27, *P*=0.001), pod length and days to flowering in winter × spring cross (-0.32, *P*=0.001), days to flowering and seeds/pod in winter × spring cross (-0.32, *P*=0.001), days to flowering and seed yield (-0.28, *P*=0.01; -0.61, *P*=0.001), root vigor and plant height in winter × spring cross only (-0.25, *P*=0.001) in spring × winter cross only.

Table 3.3. Correlation among yield, yield attributing traits and root traits in the F_2 populations of Lorenz × Legend, and Lagoda × Regent crosses.

		Spring x Winter								
		Plant Height	Branch/ plant	Pods/ Plant	Pod length	Seeds/ Pod	Days to flower	Root vigor	Root Length	Seed Yield/ plant
Winter x Spring	Plant Height	--------	**0.14***	**0.22*****	**0 .20*****	0.02	**-0.13***	**-0.25*****	0.05	0.08
	Branch/plant	**0.30*****	--------	**0.13***	**0.12***	-0.25	**-0.13***	-0.06	0.04	-0.001
	Pods/plant	**0.13***	0.09	----------	-0.02	-0.05	**-0.45***	0.03	0.06	**0.38*****
	Pod Length	**0.20*****	**0.18****	0.10	------	**0.64*****	0.03	-0.13	**0.33*****	**0.16****
	Seeds/pod	**0.14***	0.05	0.04	**0.79*****	---------	0.09	-0.08	**0.25*****	**0.30*****
	Days to flower		-0.02	**-0.27*****	**-0.32*****	**-0.32*****	--------	0.14	**0.32*****	**-0.28****
	Root Vigor	-0.04	-0.03	-0.46E-3	-0.01	0.03	**0.23***	------	0.20	0.02
	Root Length	0.05	-0.03	0.08	**0.45*****	**0.30****	**0.26*****	**0.24*****	------	**0.22*****
	Seed yield/plant		0.04	**0.32*****	**0.49*****	**0.51*****	**-0.61*****	-0.05	**0.16****	--------

3.5. Discussion

3.5.1. Root system inheritance

The root system of canola plant is a taproot that generates adventitious roots, root hair etc. outward or downward from the taproot. We have observed a wide variation of root system between the winter and spring type parents. Visually enlarged with expanded root characteristics such as basal root length, adventitious roots, root hair length, root density, and branches that contribute to large root system volume is defined as a vigorous root system (Rahman and McClean, 2012). The winter type parents have vigorous root system and spring type parents have weak root system. All the plants in F_1s and $F`_1$s for both crosses had strong vigorous root system, indicating that the vigorous root system is controlled by dominant gene(s). Rahman and McClean (2012) also reported the dominant nature of vigorous root system in canola. Three genes were identified to control the vigorous root system which was further confirmed by backcross study. This is an agreement with Rahman and McClean (2012) who first reported about the inheritance of root vigor in canola in a population derived from winter and spring type crosses.

3.5.2. Inheritance of flowering time

Flowering time inheritance was studied in the F_2 populations of four crosses. A wide variation of flowering was observed in all four crosses where the flowering started at 42 days after seeding and continued until 78 days after seeding. However, for inheritance study the segregating plants were grouped into two groups; plants that flowered and plants that did not flower. The phenotypic ratio was in consistence with 3:1 for all crosses including the pooled data indicating a monogenic inheritance of the flowering time. This observation is an agreement with Light et al. (2005) and Rahman and McClean (2012). Light et al. (2005) studied flowering time inheritance in winter and spring type crosses and found a single dominant gene is controlling

vernalization for flowering. Rahman and McClean (2012) also detected a single monogenic ratio for days to flowering. Osborn et al. (1997) detected a single major QTL for flowering time in *B. napus* corresponding to a flowering time gene in Arabidopsis. In our research, flowering time in the F_2 population varied from 41 to 78 days, whereas, average spring type parents flowered in 40 days indicating that other quantitative genes are also involved in flowering time of canola. This observation also concurs with that of Rahman and McClean (2012) for flowering time in canola. Lagercratnz et al. (1996) also reported the similar observation of effect of minor gene along with one major gene that affect the flowering time variation in *B. nigra*. Ferreira et al. (1995) identified three major QTL in *B. napus*, one of which accounted most of the flowering time variation, while the other two have only minor effects. Thurling and Das (1979) reported that two genes are responsible for different vernalization responses in *B. napus* and one of them has significantly greater effect than the other one.

3.5.3. Correlation study

In the present study, the yield-contributing characteristics, such as, pods per plant, pod length and seeds per pod were significantly correlated with yield indicating that the seed yield of canola can be improved through an increase in any of the yield contributing characteristics mentioned above. Tuncturk and Ciftici (2007), Khulbi and Pant (1999), Khan et al. (2006) and many other researchers reported similar strong correlation of the traits with yield.

Negative significant correlation was found between days to flowering and yield/plant in this study, which is similar with the findings of Malik et al. (2000) and Sandhu and Gupta (1996) in different *Brassica* species. This indicates that with increasing number of the days require for flowering, yield will be decreased. In addition, no significant positive correlation was found between days to flowering and yield contributing characteristics. It can be concluded from these

evidences that earliness might be achieved with higher yield in selection for varietal development. This statement is similar to one reached by Belete (2011) in *B. carinata*.

Significant positive correlation was detected between days to flowering and root vigor and root length which is in agreement with Rahman and McClean (2012). Thus it can be said that it would be very difficult to obtain an early flowering variety with a strong root system as the genes related to these traits might be tightly linked. However, this linkage can be broken by evaluating crossing population consists of a large number of individuals (Rahman & McClean, 2012)

In the current study, yield/plant was also significantly correlated with root length, indicating a strong effect of the root system on yield. This evidence supports the established theory stating that strong and deeper root system is able to acquire more water and nutrients from soil efficiently, which ultimately contributes to higher seed yield (Rahman & McClean, 2012). The relationship between the root length and the yield were well studied in other crops. For instance, a significant positive correlation was shown between root length and seed yield in Soybean (Brown and Scott 1984), corn (MacKay and Barber 1986) and sorghum (Jordan et al., 1983 and Sinclair, 1994).

3.6. Conclusion

Inheritance of root system and days to flowering were determined in winter × spring canola crosses. Root system is controlled by three genes whereas the days to flowering is controlled by single dominant gene. Yield attributing traits, pod length, pods/plant and seeds/pod were significantly correlated with yield/plant. Days to flowering showed significant negative

correlation with yield/plant. In addition, a strong positive correlation was found between root length and yield/plant.

3.7. References

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CHAPTER 4. QUANTITATIVE TRAIT LOCI MAPPING FOR ROOT VIGOR AND DAYS TO FLOWERING IN CANOLA (*Brassica napus* L.)

4.1. Abstract

To identify the quantitative trait loci (QTL) controlling the root vigor and days to flowering in canola (*B. napus*), a segregating F_2 population was developed from winter and spring type cross. A linkage map consisting of 262 SNP and 3 SSR markers was constructed. Two QTL, NRV-1 and NRV-2, were identified for root vigor explaining 23.5% and 15.7% of the total phenotypic variation, respectively. The left flanking marker of the QTL NRV-1 had the best hit with a *B. rapa* gene model located on chromosome A03. Two putative candidate genes, *ASA1* and *RLF* controlling root growth in *Arabidopsis*, were found close to the locus of that gene model. Two QTL, NFT-1 and NFT-2 were detected for days to flowering, accounting for 18% and 14% of the total phenotypic variation, respectively. Both the flanking markers of the NFT-1 hits *B. rapa* gene models located on chromosome A07. A flowering controlling gene *ATH1* of *Arabidopsis* was found in between of the loci containing these gene model. Additionally, blast was performed across the *B. oleracea* chromosomes and found the best hit on the chromosome C09 and C05 for both the flanking markers of root vigor NFT-1 and NFT-2, respectively. For days to flowering, NFT-1 and NFT-2, best hits were found for the flanking markers on the chromosomes C01 and C06, respectively.

Key words: Linkage map, quantitative trait loci, root vigor, days to flowering

4.2. Introduction

"Canola" (stands for "Canadian Oil Low Acid"), generally refers to the 'double low' cultivars of rapeseed species *B. rapa* and *B. napus*. Natural rapeseed oil was not well accepted for edible purpose due to high erucic acid content in, rather was popular as lubricant in the first half of the last century. Moreover, rapeseed contains high glucosinolates level in the meal, used for animal and poultry feed. As a consequence of the years of research, "Canola" was developed by Dr. Baldur Stefensson, a University of Manitoba plant breeder, in 1974 by lowering its erucic acid content (less than 2% in the oil) and glucosinolates content (less than 30 micro-mole/g in the meal). This double low canola oil is risk free for human health and well accepted by the consumers. In addition to these, canola oil contains very low amount saturated fatty acid and well-balanced polyunsaturated fatty acids (2:1 ration of linoleic acid and alpha-linolenic acid) favorable for human health (Canola Council of Canada, 2013). It is now a well-established oilseed crop throughout the world. In the United States, North Dakota alone holds 81% of the total acreage in 2012 (NASS, USDA, 2013).

The primary goal of all breeding programs is to develop new varieties by improving phenotypic and genotypic characteristics of plant which ultimately contribute greater yield. Though most morphological features of a plant are important in breeders' point of view, root characteristics were mostly overlooked by the researchers in last century. Pavlychenko (1937) first described the importance of plant roots in capturing water and nutrients which ultimately lead good crop productivity. Moreover, development of adequate root system is important for optimal plant growth; and optimal plant growth is one of the prerequisite for crop performance in terms of yield (Marschner, 1998). However, crop root traits are very complex in nature and exhibit plasticity or preferential growth to the area of high moisture and nutrients. Root growth

differs from plant to plant, even if there is small change in the soil environment. These facts together increase the difficulties in making decisions about root genetic analysis and measuring root characteristics precisely which ultimately make difficult the selection for root characteristics for the breeders. QTL mapping approach is an effective solution to understand the genetic basis of root morphological traits which could be used for marker assisted selection in breeding program.

Studies available on root characteristics in canola are very limited. Root architecture of canola varies greatly with different habitats; spring canola possesses a light root system whereas winter canola has vigorous and dense root system. As there is a high genetic diversity among the germplasms of different habitats (Rahman, 2013), winter canola can serve as a useful source of genetic diversity to improve spring canola root architecture. Rahman and McClean (2012) first reported that root vigor in canola is controlled by three genes. QTL analysis on root related morphology have been conducted in Brassica species by only few researchers, such as Lu et al. (2008) in *B. rapa*, Lei et al. (2012) in *B. napus*, Yang et al. (2010) in *B. napus*. QTL analysis was performed for different root traits in other crops including rice (Tuberosa et al., 2002; Yadav et al., 1997), in maize (Lebreton et al., 1995; Guingo et al., 1998; Zhu et al., 2006).

Flowering time in canola also varies greatly with different habitats. Early flowering spring canola flowers without vernalization, whereas late flowering winter canola needs vernalization for flowering. Lagercantz et al. (1996) stated the importance of modification of flowering time in Brassica crops for expanding the geographical range of cultivation. For example, early flowering is a common trait of interest among the canola breeders in Canada to expand the canola cultivation further north (Murphy and Scarth, 1994). Many QTL for flowering time (explaining both large and small phenotypic variation) have been identified so far in

different *Brassica* sp. Ferreira et al. (1995) identified three QTL in *B. napus*, one of which is describing the most flowering time variation while the other two have a minor effect only. Osborn et al. (1997) reported that this largest QTL effect (explaining 50% of total phenotypic variation) of *B. napus* is related to a major vernalization-responsive flowering time gene (*VFN2*) of Arabidopsis. Delourme et al. (2006) performed QTL analysis for earliness of flowering in two double haploid populations derived from *B. napus* and detected three and nine QTL, respectively. Quijada et al. (2006) identified ten QTL for days to flower. Lou et al. (2007) evaluated several *B. rapa* populations under different environmental conditions and identified a major QTL corresponding to *B. rapa Flowering Locus C* (*BrFLC2*) gene located on linkage group A02. Zhao et al. (2010) identified three flowering time QTL and two vernalization responsive QTL in two linkage groups. In *B. napus,* co-localization of flowering time QTL with yield and yield-related QTL has also been reported in other studies (Udall et al., 2006; Basunanda et al., 2010; Chen et al., 2010). Two QTL associated with both flowering time and flowering-time index and one additional QTL for flowering-time index only were identified by Camargo et al. (1996) in *B. oleracea.* However, very few of the above mentioned studies was carried out in biparental F_2 population derived from winter and spring type crosses. The objective of this study was to perform QTL analysis for root vigor and days to flower in F_2 population derived from winter and spring type canola. Additionally, use homology based searches with *B. rapa* and *B. oleracea* genomes to identify the candidate genes for these traits.

4.3. Materials and methods

4.3.1. Plant materials

The spring type canola cultivar ‘Regent’ (PI431572) and winter type cultivar ‘Lagoda’ were obtained from USDA-ARS National Plant Germplasm System and University of Alberta,

Canada, respectively. The parents were crossed (F_1) and reciprocally crossed (F'_1) with each other. The F_1s and F'_1s were then advanced to the F_2 generation. The F_1, F'_1, and F_2 populations were grown in the greenhouse at 23±1°C (day and night). The plants in the greenhouse were provided with a 16-h photoperiod provided by natural sunlight supplemented with 400 W HPS PL 2000 lights (P.L. Light Systems Inc. ON, Canada). The seeds were sown in 6-inch (diameter) x 6-inch (depth) pots filled with Sunshine-Mix-1 (Sun Gro Horticulture, BC, Canada). Plants were watered daily and fertilized with water-soluble 20-20-20 fertilizer.

4.3.2. Phenotype

Root vigor was measured at approximately 50% of flowering on the basis of visual scoring on a scale of 1-5, according to Rahman and McClean (2012), where, score-1 has weak bottom and surface roots, those observed in spring-type parents, score-2 has more bottom and surface roots, score-3 has intermediate bottom and surface roots, score-4 has strong bottom and surface roots, and score-5 has the strongest bottom and surface roots, those found in winter-type parents (Fig 4.1). Days to flowering was recorded from seeding time to the first open flower. Data were taken every day, whenever a plant flowered, up to 76 days from planting. The plants that were not flowered within this period regarded as "not flowered".

Figure 4.1. Root Vigor score on a scale of 1-5 (left to right) (1= weak bottom and surface roots, those observed in spring-type parents, 2 = more bottom and surface roots, 3 = intermediate bottom and surface roots, 4 = strong bottom and surface roots, and 5 = the strongest bottom and surface roots, those found in winter-type parents)

4.3.3. DNA isolation and marker analysis

Young leaves were collected for DNA isolation when plants reached the four leaf stage. DNA was isolated from these young leaves by using CTAB method (Murray and Thompson 1980) for SSR marker screening. In addition, DNA was also isolated by using Qiagen DNA Extraction kit following manufacturer's instruction. These pure DNA were sent for genotyping-by-sequencing (GBS) to the Institute of Genomic Diversity, Cornell University, Ithaca, NY. GBS libraries were prepared and analyzed at the Institute for Genomic Diversity (IGD), according to Elshire et al. (2011), using the enzyme *ApeK1* for digestion and create a library with 95 unique barcodes. The GBS UNEAK analysis pipeline [Tassel Version: 3.0.160] (Lu et al 2013), an extension to the Java program TASSEL (Bradbury et al., 2007), was used to call SNPs from the sequenced GBS library. VCFtools (v0.1.10) (Danecek et al., 2011) was used to summarize and filter data. In addition, 12 SSR markers from different chromosomes were also screened to the same population (Cheng et al., 2009).

4.3.4. Linkage map construction

Markers that are polymorphic between the parents were used in further analyses. Markers and the genotypes that have more than 50% of missing data were excluded. Chi-square goodness of fit test was performed across the remaining markers to check the ratio of 1:2:1 as expected in F_2 population and those were removed that did not fit with the ratio at a significance level of 0.01. Polymorphic markers were used to construct the linkage map in Carthagene (de Givry et al. 2005*)* for the F_2 population. At first, "group" command was used to determine the linkage groups with a recombination frequency of 0.4 and LOD score 9. Framework map for each linkage groups were further constructed by using "buildfw" command with an "adding and keeping threshold" of 5. "Flips" command was used to select the best map by testing all possible

permutation of the markers. The reliability of the maps was further verified by swapping pairs of markers with the 'polish' command. Kosambi mapping function (Kosambi, 1944) was used as a measure of recombination frequency.

4.3.5. QTL detection

QTL analysis was performed by using the genetic map and the phenotype data with composite interval mapping method (CIM) (Zeng, 1994) in Qgene 4.3.10 (Joehanes and Nelson, 2008). Cofactors were selected using the stepwise selection procedure. A default walking distance of 2 cM was used for the analysis. A QTL is defined as the region on chromosome that has a LOD ≥ 3. In addition, a single marker analysis was conducted for unlinked markers to associate markers with the phenotype. The additive effect and percentage of phenotypic variance explained by each QTL were also estimated.

4.3.6. Blast analysis

The tag sequences of the significant markers were blasted against *B. rapa* 1.5 peptide sequences available at http://www.brassica.info. The cut-off criteria used was an e-value of 1E-10 and a 50% minimum query length with at least 50% identity. In addition, they were blasted against the TAIR 10 database of *Arabidopsis* to annotate the genes linked to for the phenotypic variation in canola. If there is no significant marker on the QTL, the flanking markers of the QTL region, were used to search for candidate genes that control a phenotypic variation in *B. rapa*. Candidate genes were obtained by searching the TAIR website with the appropriate words of genes. Additionally, we performed blast across the *B. oleracea* v 1.0 chromosomes available at http://www.ocri-genomics.org/bolbase/blast/blast.html

4.4. Results

4.4.1. Linkage map

A total of 11,244 SNP markers were obtained for the population and only 935 markers were found polymorphic between the two parents. The final linkage map was constructed with 262 SNP and 3 SSR markers assigned onto 26 linkage groups. These 26 linkage groups cover a total of 2469 cM. The length of linkage groups were between 9.6 cM to 266.8 cM.

4.4.2. QTL analysis of root vigor

Two QTL (NRV-1, NRV-2) were detected for root vigor. The first QTL NRV-1 was located on LG25 at 22 cM and was significant at a LOD of 5.51. The second QTL NRV-2 was located on LG5 at 74 cM at a significant LOD of 3.38. NRV-1 and NRV-2 explain a phenotypic variation of 23.5% and 15.5%, respectively (Table 4.1). For the NRV-1, the left flanking marker S1_54737009, which is 2.5 cM distant from the QTL peak, had a best hit with the *B. rapa* gene model Bra023958 located on chromosome A03 at 28.63 Mbp. Two candidate genes, *ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1* (AT5G05730.1) and *REDUCED LATERAL ROOT FORMATION* (AT5G09680.2) were found at 28.86 Mbp and 28.21 Mbp away from the left flanking marker S1_54737009, respectively. However, the right flanking marker S1_57154030 had no hit in the *B. rapa* genome. No significant hit was found for the flanking markers of the second QTL NRV-2. Other than these two QTL, three markers designated as S1_42763025, S1_54665025, S1_151062 with LOD $\geq$ 3.00 were not assigned in any LG. No significant BLAST hit were found for these single markers too. However, the flanking markers of both QTL have a hit with the *B. oleracea* chromosomes. The flanking markers of NRV-1 hits on the chromosome C09 whereas, the flanking markers of NRV-2 have hits with the chromosome C05.

4.4.3. Days to flowering

Two flowering time QTL, NFT-1 and NFT-2, were identified with a LOD of 3.88 and 3.06, respectively. The QTL NFT-1 is located on LG 17 at 182 cM and explains 18% of the phenotypic variation, whereas, the NFT-2 is located on LG 26 at 132 cM and explains 14% of phenotypic variation. The left flanking marker S1_4586031 of NFT-1 had a blast hit with the *B. rapa* gene model Bra002005 located on A07 at 26.66 Mbp. The right flanking marker S1_31008060 had a significant hit with gene model Bra002098 located on A07 at 18.55 Mbp. A candidate gene for flowering time, *HOMEOBOX GENE 1* was found within this QTL at 23.31 Mbp. However, no significant blast hit was detected for the flanking markers of the other QTL NFT-2. Additionally three more markers S1_1018039, S1_5404018 and S1_39838049 were found having LOD value greater than 3.0. The latter two single markers have a hit with the *B. rapa* gene model Bra007391 located on chromosome A09 at 30.48 Mbp. A candidate gene *MITOTIC-LIKE CYCLIN 3B* was found on the same locus. The flanking markers of NFT-1 have a hit on chromosome C01 when BLAST was performed across *B. oleracea* chromosomes. Similarly, significant hits on chromosome C06 were found for both of the flanking markers of NFT-2.

Table 4.1. Characteristics of identified QTL affecting root vigor and days to flowering in F_2 population of "Lagoda" and "Regent" crosses.

Trait	QTL	LG	Additive effect	LOD	R-square	Markers		Putative Chromosome (*B. rapa*)	Putative Chromosome (*B. oleracea*)
						Left Flanking	Right Flanking		
Root Vigor	NRV-1	25	-1.76	5.31	23.5	S1_54737009	S1_57154030	A03	C09
	NRV-2	5	-0.08	3.38	15.7	S1_45383063	S1_35705018	-	C05
Days to Flowering	NFT-1	17	1.27	3.89	18	S1_4586031	S1_31008060	A07	C01
	NFT-2	26	3	3.06	14	S1_37194020	S1_59952011	-	C06

4.5. Discussion

4.5.1. Root vigor

Most of the scientific approaches and breeding selections to increase the crop yield in the last century were focused on above ground plant parts and soil nutrient management. However, the below ground plant part, root, is also equally important and plays a vital role in contributing yield. Root vigor enhances root growth by promoting root formation in all direction, developing a strong root system which ultimately supports the canopy aboveground. Based on this importance, our current study is focused to identify quantitative trail loci (QTL) related to root vigor in canola. A trigenic inheritance was identified controlling the root vigor after analyzing the phenotype of the mapping population of 90 F_2 individuals derived from winter and spring type canola. This is in accordance with our earlier findings on root vigor in a bigger population. Rahman and McClean (2012) also reported three genes controlling root vigor in *B. napus*. However, we identified two QTL, NRV-1 with LOD value 5.31 explaining 23.5% of total phenotypic variation, and NRV-2 with LOD value 3.38 explaining 15% of the total phenotypic

variation for root vigor. Until now, very few researches were carried out to identify quantitative trait loci for root characteristics in *Brassica* sp. Moreover, most of these studies were aimed to identify QTL for root traits in response to differential nutrient condition of soil or to drought. Lu et al. (2008) first performed QTL analysis for different root morphological traits in *B. rapa* and identified 7 QTL for taproot thickness, 5QTL for taproot length and 6 QTL for taproot weight. However, recently, Lei et al. (2012) identified total 8 QTL for increment of primary root number for both low Boron (4 QTL) soil and optimum Boron soil (4 QTL) which they assigned on the chromosome A01, A02, A05, C03 and C04. In addition, they also identified total 5 QTL (2 in low B soil and 3 in optimum B soil) for root dry weight and assigned them on A02, C04, C07 and C09 chromosomes.

In the blast analysis, the left flanking marker of NRV-1 hits the *B. rapa* gene model on chromosome A09. Two putative candidate genes, *RLF* (*REDUCED LATERAL ROOT FORMATION*) and *ASA1* (*ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1*), were found near by the locus of this flanking marker. *RLF* gene is a cytosolic protein involved in early cell division in root which initiate the lateral root formation (Ikeyama et al., 2010). *ASA1* is a well characterized auxin biosynthetic gene that is required for ethylene-induced auxin production, and therefore plays a role in ethylene-mediated regulation of root growth inhibition (Stepanova et al., 2005, Sun et al., 2009).

In addition, we performed BLAST across the *B. oleracea* chromosomes and found that the flanking markers of NRV-1 hit the chromosome C09. This result showed a partial agreement with Lei et al. (2012) who identified root dry weight QTL located on chromosome C09. In case of NRV-2, both the right and left flanking markers hit the chromosome C05.

4.5.2. Days to flowering

A few studies on QTL mapping for days to flower have been reported in *Brassica* species by using the population derived from the winter and spring type crosses. We used the F_2 population derived from the cross between vernalization requiring winter type variety "Lagoda and no vernalization required early flowering spring type variety "Regent". A continuous flowering time variation was observed in the F_2 segregating population that started at 42 days after seeding and continues to 78 days after seeding. Different flowering controlling genes may have various level of contribution to flowering, and recombination of those genes may result a wide variation of flowering time in the F_2 population. Many quantitative trait loci for flowering time (both large and small effect) have been identified so far in different *Brassica* sp. In the current study, we identified two QTL, NFT-1 (LOD 3.88 and explains 18% phenotypic variation) and NFT-2 (LOD 3.06 and explains 14% phenotypic variation), responsible for days to flowering. Three major QTL in *B. napus* were detected by Ferreira et al. (1995), one of which showed the major effect and the other two showed minor effect on days to flowering. Delourme et al. (2006) detected three and nine QTL responsible for earliness of flowering in two different DH population derived from *B. napus*. Long et al. (2007) reported huge variation of flowering time at 11 field environments and detected 5-18 QTL in each environment. Chen et al. (2010) identified 22 flowering time QTL in *B. napus* explaining 4.41%–48.28% of the total phenotypic variance.

Although many QTL were identified for flowering time in *Brassica*, very few of them have been assigned on chromosome. For QTL assignment on chromosome, a blast analysis was performed on *B. rapa* genome by using the SNP tag sequences. Both the left and right flanking markers of QTL NFT-1 hit the chromosome A07 of *B. rapa* gene model. A candidate gene locus

containing *HOMEOBOX GENE-1,* responsible for flowering in Arabidopsis, was found in between these two flanking marker loci. Assignment of flowering time gene on chromosome A07 showed an agreement with Mei et al. (2009) and Udall et al. (2006) who identified flowering time QTL located on *B. napus* chromosome N07 (A07). In *B. rapa*, several QTL (*VFR1, VFR2, VFR3* and *FR1, FR2, FR3*) were identified in a F_2 and RIL population (Teutonico and Osborn, 1994, Osborn et al. 1997, Schranz et al. 2002, Lou et al. 2007). Among them, *VFR2* is located at the *BrFLC1* locus, *FR1* is located at *BrFLC2* locus and *FR2* is located on *BrFLC5* locus (Schranz et al. 2002). The four *B. rapa* flowering time gene, *BrFLC1, BrFLC2, BrFLC3* and *BrFLC5* were later assigned to chromosome A10, A02, A03 and A03 respectively (Kole et al., 2001, Schranz et al., 2002; Kim et al., 2006; Yang et al., 2006; Lou et al., 2007; Zhao et al., 2010). In addition, Zhao et al. (2007) identified 3 markers associated with days for flowering in vernalized *B. rapa* that are distributed on chromosome A02, A03 and A05.

The putative candidate gene that identified between the two loci, related to the QTL NFT-1 flanking markers, is annotated as *Arabidopsis thaliana HOMEOBOX GENE -1 (ATH-1).* This gene was originally isolated for light-regulated transcription factors (Quaedvlieg et al., 1995). Proveniers et al. (2007) hypothesized that *ATH-1* may have a role in activation of Arabidopsis flowering time gene *Flowering Locus C* (*FLC*). *FLC* is regarded as the most prominent floral repressor in Arabidopsis (Michaels and Amasino, 1999; Sheldon et al., 1999). Proveniers et al. (2007) investigated the function of *ATH1* using expression analysis and reverse genetics approach and showed *ATH1* is a specific activator of *FLC* and control the floral competency. In addition, there are evidences that different kinds of *Homeobox genes* play a significant role in floral development and expression in different crops such as, sunflower (Rueda et al. 2005), rice (Sentoku et al., 1999), and tobacco (Uberlaker et al., 1996).

In addition to QTL for flowering time, three single markers having LOD < 3.00 related to flowering time were also identified. Two of them hit the *B. rapa* gene model on chromosome A09 at a locus that contains *MITOTIC-LIKE CYCLIN 3B* gene. *Cyclin* is a large group of genes; of which B type cyclin genes are highly expressed in roots, flowers and meristematic region and leaves (Day and Reddy, 1998). Among the B-type cyclin genes (*Cyclin 1B, Cyclin 2B* and *Cyclin 3B*), the *Cyclin 3B* expressed in a high level in anther and inflorescence, and less in leaves and roots (Wang et al., 2004).

BLAST analysis on the flanking markers sequences was conducted across the *B. oleracea* chromosomes and assigned them on different chromosomes based on the best hits. Both the left and right flanking markers of NFT-1 have hits on chromosome C01. On the other hand, flanking markers of NFT-2 have best hits on the chromosome C06. This result showed an agreement with Rae et al. (1999) who identified two QTL, one for early flowering and the other one for late flowering in a population of recombinant backcross substitution lines derived from cross between two *B. oleracea* germplasm and mapped both of them on chromosome C01 close to each other. Okazki et al. (2007) detected a minor non-significant QTL for flowering time on chromosome C06.

This is the first molecular approach to explore the complex genetics behind the root architecture in canola. However, this study was carried out in the controlled environment in greenhouse. Further research should be conducted in field conditions to determine the nature of genetics of root characteristics more accurately. In this study, we identified the quantitative trait loci for root vigor and further studies on related traits like tap root length, root dry weight, primary root number are warranted. The major limitation to this study is identifying the precise location of the QTL, given that there are several hits in Blast for the same marker. We assume

that this is due to the mesohexaploid nature of the diploid *B. rapa* species caused by Whole Genome Triplication (WGT) that occurred millions of years ago (Cheng et al., 2013). We also assigned our QTL on tentative chromosomes of *B. oleracea* based on the best BLAST hits. We couldn't identify any candidate genes on C genome because the whole C genome sequence is not yet available. However, this study can be regarded as the first step towards our ultimate goal to marker assisted selection for the root morphology in canola.

4.6. Conclusion

Quantitative trait loci (QTL) were identified for root vigor and days to flowering in canola (*B. napus*) in a F_2 population derived from winter type and spring type cross. A linkage map was constructed with 262 single nucleotide polymorphism (SNP) and 3 single sequence repeat (SSR) markers. Two QTL for root vigor, NRV-1 and NRV-2, were identified explaining 23.5% and 15.7% of the total phenotypic variation, respectively. Two putative candidate genes on chromosome A03, *REDUCED LATERAL ROOT FORMATION (RLF)* and *ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1* (*ASA1*) controlling root growth in Arabidopsis, was detected based on blast analysis. Two QTL, NFT-1 and NFT-2 were detected controlling days to flowering accounting for 18% and 14% of the total phenotypic variation, respectively. A flowering time controlling gene *HOMEOBOX GENE -1* of Arabidopsis was found in between of the loci containing the gene models that have blast hit for the flanking markers of NFT-1. Best blast hits were found on the *B. oleracea* chromosome C09, C05, C01 and C06 for the flanking markers of NRV-1, NRV-2, NFT-1 and NFT-2, respectively.

4.7. References

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APPENDIX

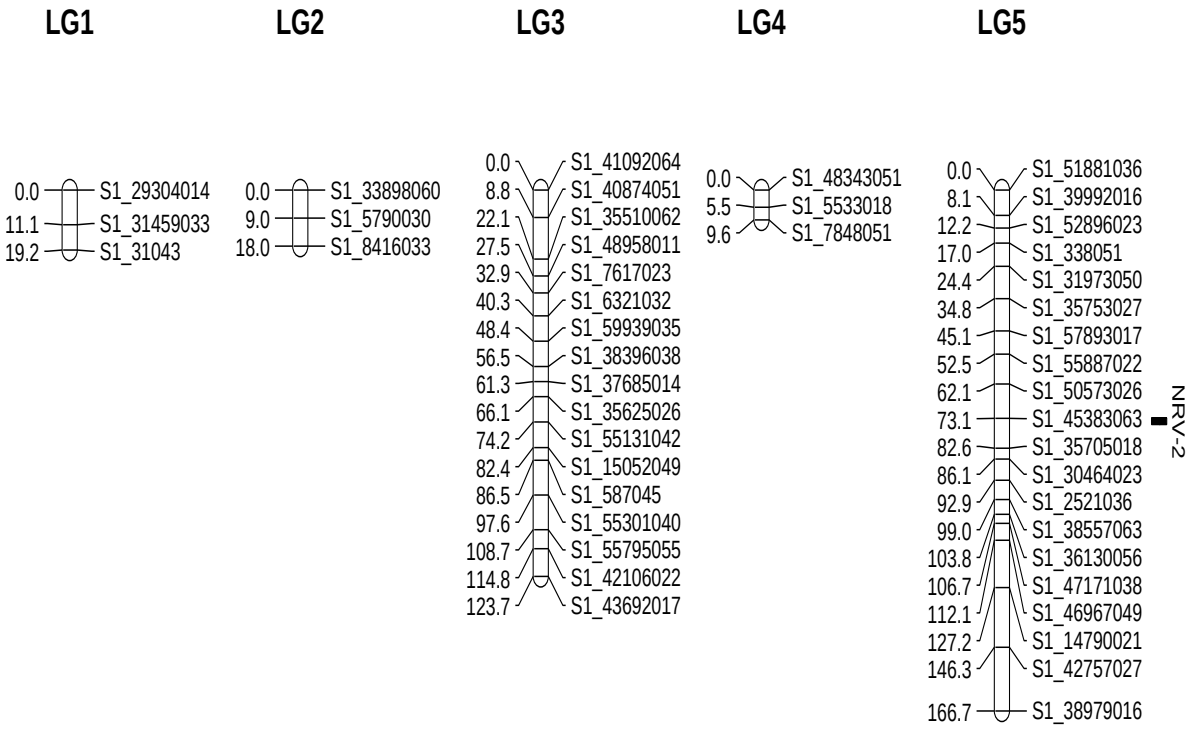


Figure A.1. Linkage groups with 262 single nucleotide polymorphism (SNP) and 3 single sequence repeat (SSR) markers.

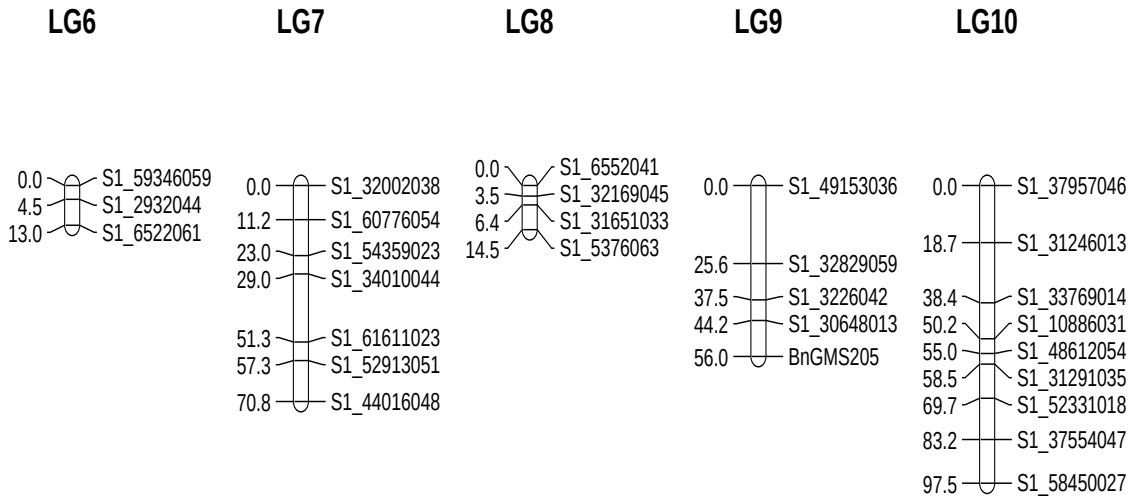


Figure A.1. Linkage groups with 262 single nucleotide polymorphism (SNP) and 3 single sequence repeat (SSR) markers (continued).

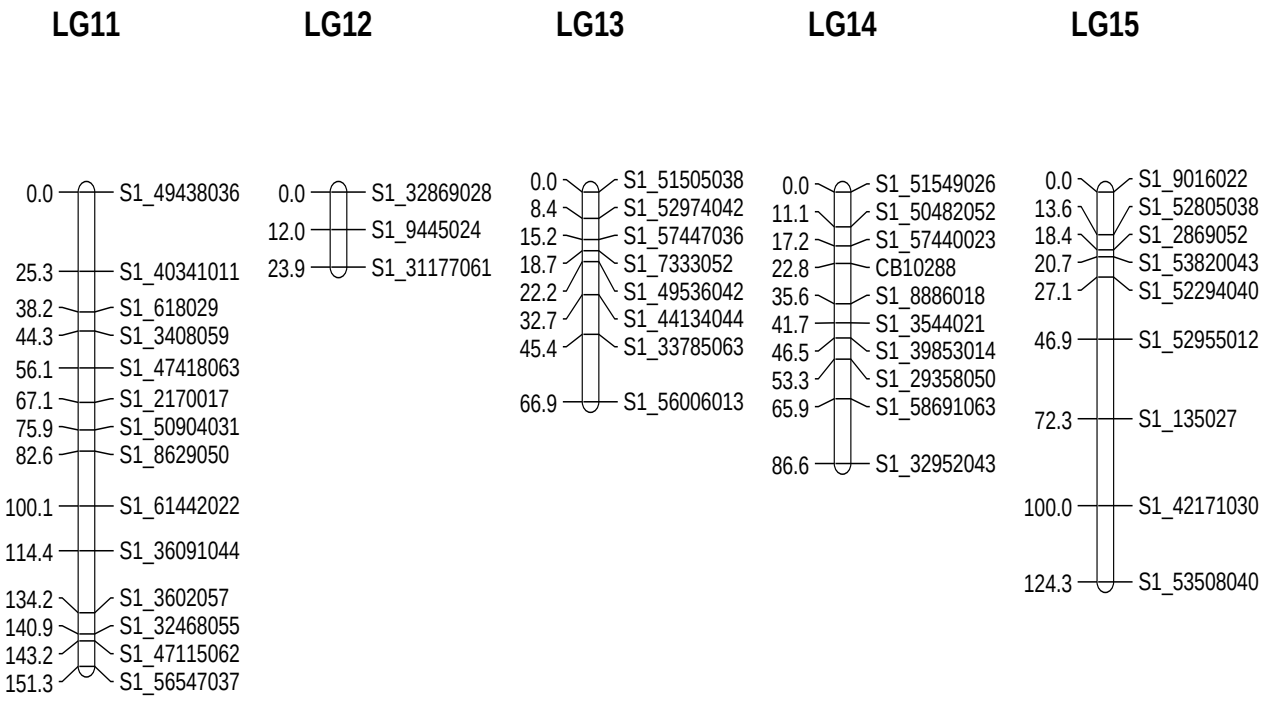
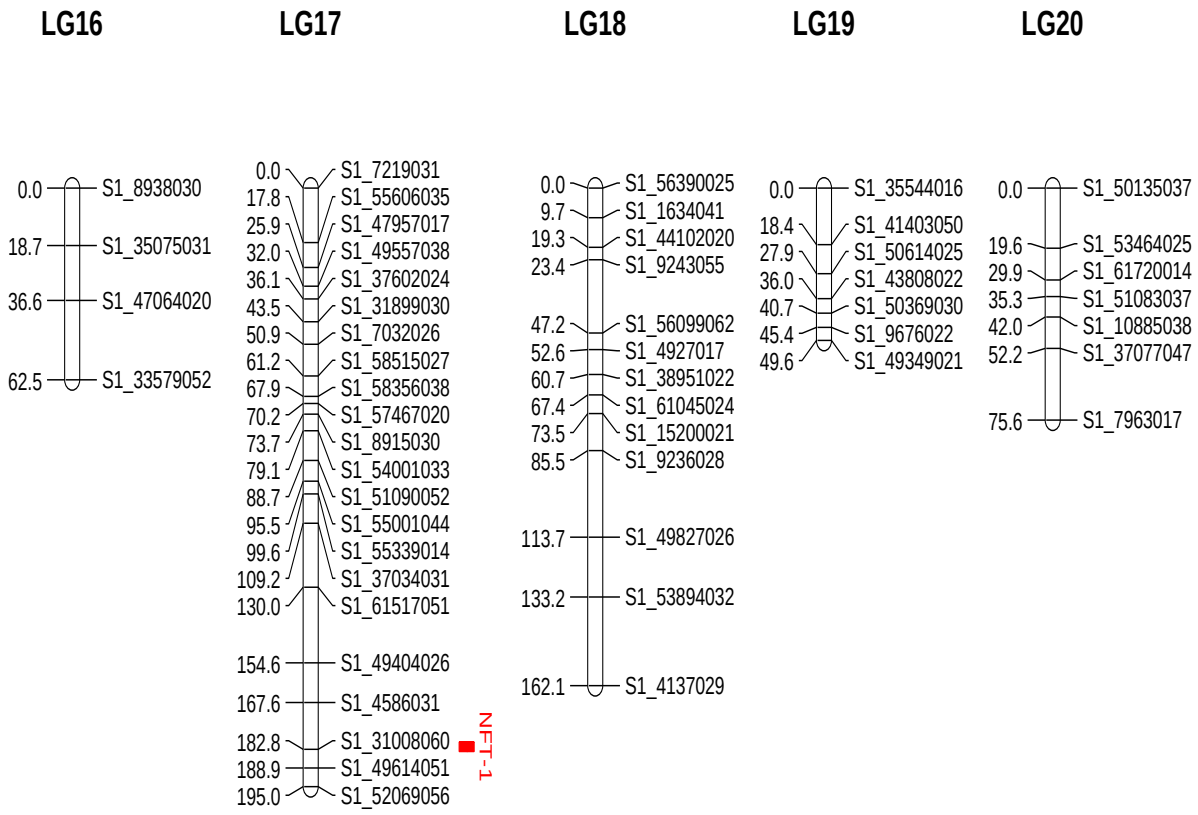


Figure A.1. Linkage groups with 262 single nucleotide polymorphism (SNP) and 3 single sequence repeat (SSR) markers (continued).



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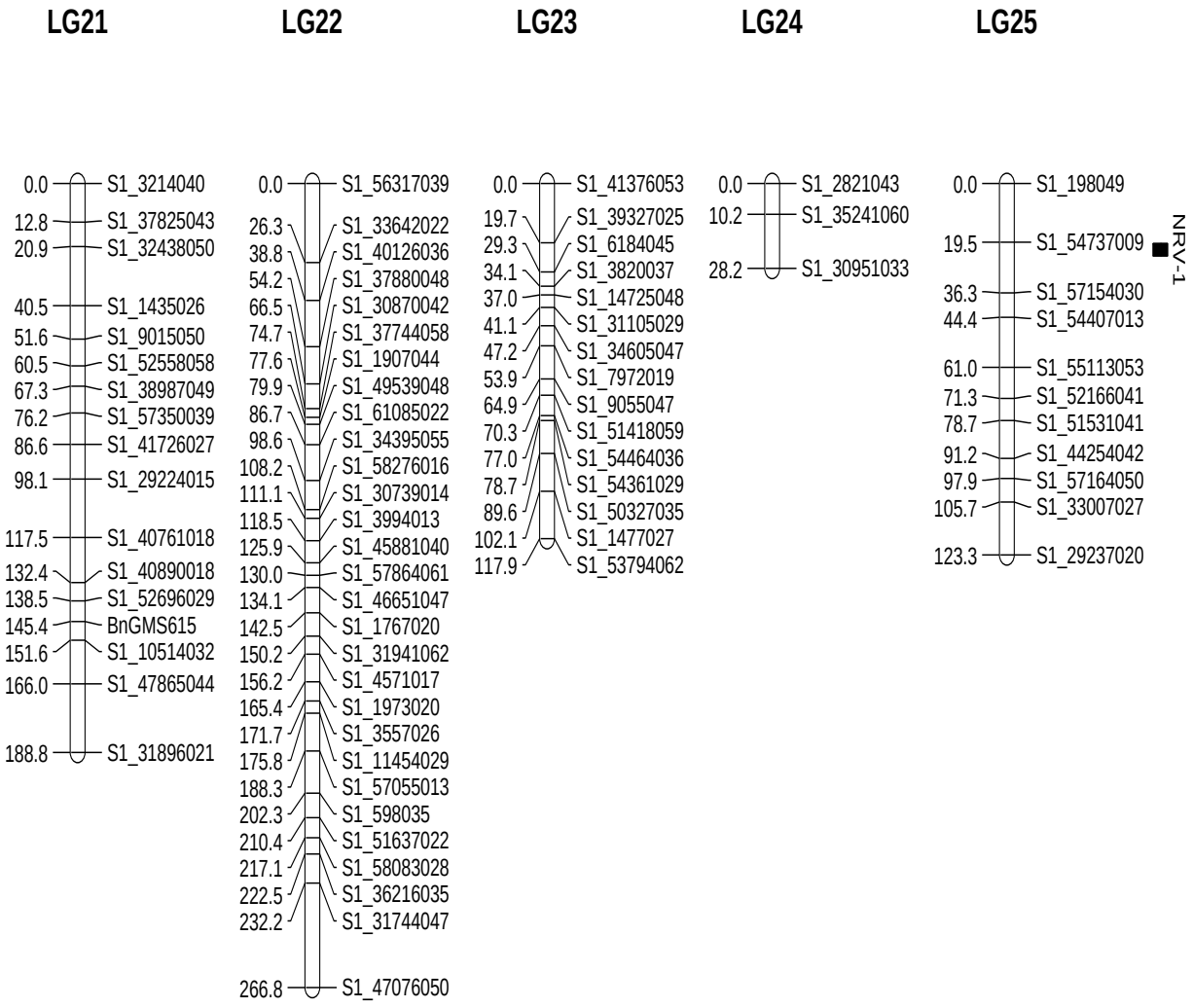

Figure A.1. Linkage groups with 262 single nucleotide polymorphism (SNP) and 3 single sequence repeat (SSR) markers (continued).

LG26

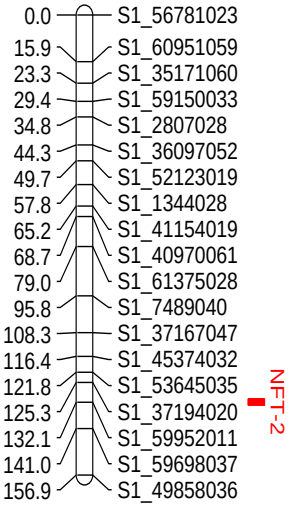

Figure A.1. Linkage groups with 262 single nucleotide polymorphism (SNP) and 3 single sequence repeat (SSR) markers.

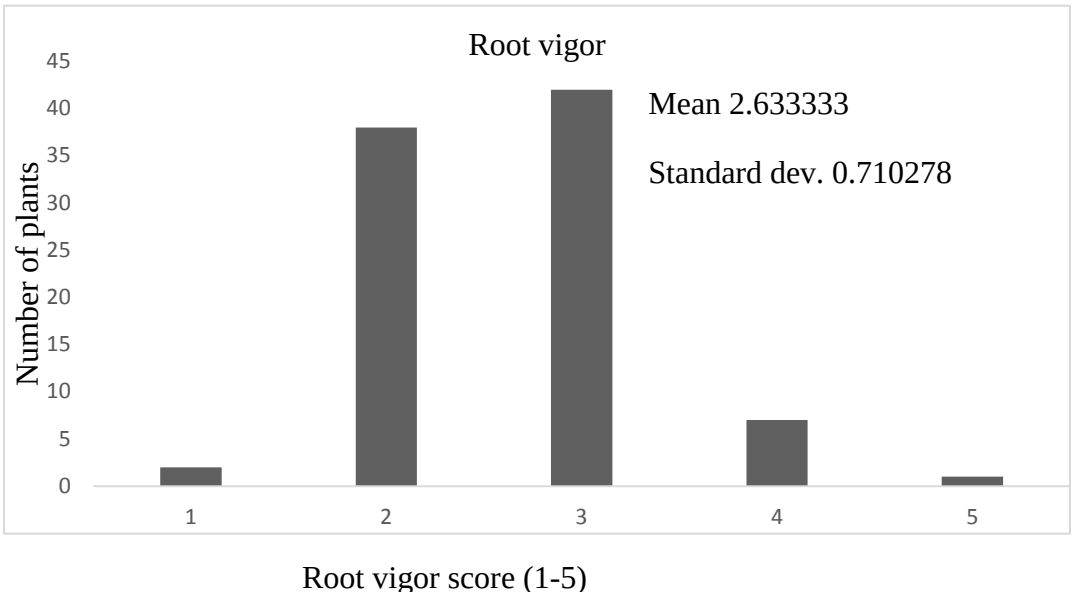

Figure A.2. Phenotypic distribution of root vigor on a scale 1-5 of 90 F_2 plants.

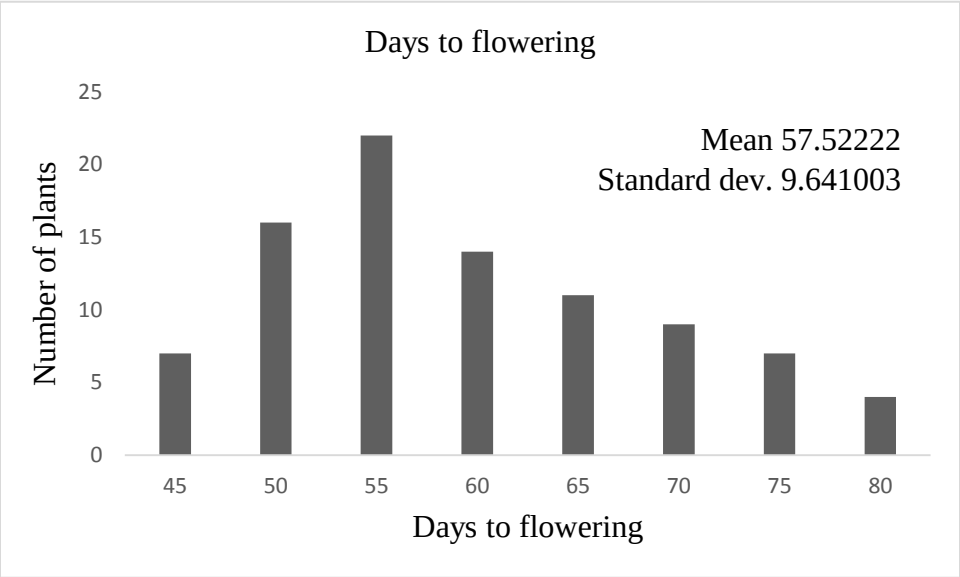

Figure A.3. Phenotypic distribution of days to flowering of 90 F_2 plants.

Table A.1. List of SSR markers with sequences

Name	Linkage Group	Chromosome	Left Sequence	Right Sequence
BnGMS615	21	N5	GGCACGGATCTTAACCTAGT	TCAAGGGCTTTCATATCTTG
BnGMS205	9	N16	AAGAGAGACAGCGTGTTGTT	GACTCTAGGAGAGTAGACGGC
CB10288	14	N14	GCAATGCATATCGACCTT	AACCGCGCTATCAAGAAT

Table A.2. Blast analysis with *B. rapa* genome for the flanking markers of the identified QTL and the significant single markers and the putative candidate genes with their position

Trait	QTL	QTL Position in LG (cM)	Flanking Markers	Position in LG (cM)	Gene Model	Putative Chr	Position in Chr (Mbp)	Putative Candidate gene	Candidate gene Position (Mbp)
Root Vigor	NRV-1	22	S1_54737009 (L)	19.5	Bra023958	A03	28.63	RLF and ASA1	28.21 and 28.86
			S1_57154030 (R)	36.3	No hit	N/A	--	--	--
	NRV-2	74	S1_45383063 (L)	73.1	No hit	N/A	--	--	--
			S1_35705018 (R)	82.6	No hit	N/A	--	--	--
	Single markers		S1_42763025	N/A	No	N/A	--	--	--
			S1_54665025	N/A	No hit	N/A	--	--	--
			S1_151062	N/A	No hit	N/A	--	--	--
Days to Flower	NFT-1	182	S1_4586031 (L)	167.6	Bra002005	A07	26.66	Homebox gene 1	23.31
			S1_31008060 (R)	182.8	Bra002098	A07	18.55		
	NFT-2	132	S1_37194020 (L)	125	No hit	N/A	--	--	--
			S1_59952011 (R)	132	No hit	N/A	--	--	--
	Single markers		S1_1018039	N/A	No hit	N/A	--	--	--
			S1_5404018	N/A	Bra007391	A09	30.48	Mitotic-like Cyclin 3B	N/A
			S1_39838049	N/A	Bra007391	A09	30.48	Mitotic-like Cyclin 3B	N/A