INHERITANCE AND QTL IDENTIFICATION OF SEED OIL, LEAF CHLOROPHYLL AND ANTHOCYANIN CONTENTS IN RAPESEED (*Brassica napus* L.)

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การถ่ายทอดลักษณะและระบุตำแหน่งของเครื่องหมายโมเลกุลที่สัมพันธ์กับยีนที่ควบคุมลักษณะปริมาณน้ำมันในเมล็ด ปริมาณคลอโรฟิลล์และแอนโทไซยานินในใบของเรพซีด (*Brassica napus* L.)

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาการถ่ายทอดลักษณะที่ควบคุมปริมาณน้ำมันในเมล็ด ปริมาณคลอโรฟิลล์และแอนโทไซยานินในใบของเรพซีด รวมถึงระบุตำแหน่งของเครื่องหมายโมเลกุลที่สัมพันธ์กับยีนเชิงปริมาณที่ควบคุมลักษณะดังกล่าว โดยทำการศึกษาเรพซีด 6 ประชากร (P_1 P_2 F_1 F_2 B_1 และ B_2) จากคู่ผสมระหว่างสายพันธุ์แท้ 2 สายพันธุ์คือ 10-Zi006 (ใบสีม่วง) กับ 10-4438 (ใบสีเขียว) วางแผนการทดลองแบบสุ่มบล็อกสมบูรณ์เป็นเวลา 2 ปี จากนั้นตรวจวัดการแสดงออกของยีนและการถ่ายทอดลักษณะน้ำมันในเมล็ด ปริมาณคลอโรฟิลล์และแอนโทไซยานินในใบ พบว่า การถ่ายทอดลักษณะที่ศึกษาไม่ได้เป็นไปตาม additive-dominance genetic model แสดงให้เห็นว่าผลของปฏิกิริยาสัมพันธ์ระหว่างยีนต่างอัลลีลมีความสำคัญกับลักษณะที่ตรวจวัด จากการทดลองยังพบว่าปริมาณแอนโทไซยานินมีค่าความสามารถในการถ่ายทอดทางพันธุกรรมแบบกว้างสูงและมีความสามารถในการถ่ายทอดทางพันธุกรรมแบบแคบปานกลางแสดงให้เห็นว่าปริมาณแอนโทไซยานินถูกควบคุมด้วยพันธุกรรมเป็นหลักด้วยยีนแบบผลบวกและแบบที่ไม่ใช่ยีนแบบผลบวก และยังพบว่าปริมาณน้ำมันในเมล็ดและปริมาณคลอโรฟิลล์มีค่าความสามารถในการถ่ายทอดทางพันธุกรรมแบบกว้างปานกลางและมีความสามารถในการถ่ายทอดทางพันธุกรรมแบบแคบต่ำแสดงให้เห็นว่าทั้งสองลักษณะนี้ถูกควบคุมทั้งจากพันธุกรรมและสภาพแวดล้อม และยีนแบบผลบวก ยีนแบบที่ไม่ใช่ยีนแบบผลบวก และสภาพแวดล้อมมีความสำคัญต่อการแสดงออกของลักษณะ จากการศึกษายังพบความสัมพันธ์ระหว่างปริมาณคลอโรฟิลล์และแอนโทไซยานินในใบที่แตกต่างกันในแต่ละประชากรด้วย

จากการจำแนกข้อมูลทางพันธุกรรมของเรพซีดทั้งจีโนมด้วยใช้เทคนิค DArT-seq พบว่าสามารถสร้างแผนที่ทางพันธุกรรมที่มีตำแหน่งเครื่องหมายโมเลกุลที่ต่างกัน 1614 ตำแหน่งจากเครื่องหมายโมเลกุลทั้งหมด 9212 เครื่องหมาย โดยพบตำแหน่งของเครื่องหมายโมเลกุลที่สัมพันธ์กับยีนเชิงปริมาณที่ควบคุมลักษณะ (Quantitative trait loci; QTLs) ปริมาณน้ำมันในเมล็ด ปริมาณคลอโรฟิลล์และแอนโทไซยานินในใบ 11, 14 และ 8 QTLs ตามลำดับ ซึ่งสามารถอธิบายความแปรปรวนของฟีโนไทป์ได้ 1.99-18.30, 9.17-19.13 และ 4.45-13.07 เปอร์เซ็นต์ ตามลำดับ จากการทดลองยังสามารถระบุตำแหน่งของเครื่องหมายโมเลกุลที่สัมพันธ์กับยีนบนโครโมโซม A06 ที่ทับซ้อนกันที่ควบคุมลักษณะน้ำมันในเมล็ดและปริมาณคลอโรฟิลล์ในใบ นอกจากนี้ยังพบว่าตำแหน่ง

ของเครื่องหมายโมเลกุลที่สัมพันธ์กับยีนเชิงปริมาณที่ควบคุมลักษณะส่วนใหญ่สอดคล้องกับแผนที่พันธุกรรมทางกายภาพในช่วง 0.02 ถึง 14.54 Mbp ซึ่งผลการศึกษานี้เป็นประโยชน์อย่างยิ่งในการหาตำแหน่งของยีนที่สนใจจากแผนที่โครโมโซม รวมถึงการใช้เครื่องหมายโมเลกุลเพื่อช่วยในการคัดเลือกลักษณะปริมาณน้ำมันในเมล็ด ปริมาณคลอโรฟิลล์และแอนโทไซยานินในใบของเรพซีด

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2557 ลายมือชื่ออาจารย์ที่ปรึกษา_____

DAI WENDONG : INHERITANCE AND QTL IDENTIFICATION OF SEED OIL, LEAF CHLOROPHYLL AND ANTHOCYANIN CONTENTS IN RAPESEED (*Brassica napus* L.). THESIS ADVISOR : TEERAYOOT GIRDTHAI, Ph.D., 212 PP.

RAPESEED/OIL CONTENT/ANTHOCYANIN CONTENT/CHLOROPHYLL CONTENT/INHERITANCE/GENETIC LINKAGE MAP/QTL/

This research aims to study the inheritance of seed oil, leaf chlorophyll and anthocyanin contents in rapeseed and to identify the quantitative trait loci (QTL) based on the high density genetic linkage map. Two inbred lines of 10-Zi006 (purple red leaf) and 10-4438 (green leaf) were employed to construct six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2). Gene action and heritability of seed oil, leaf chlorophyll and anthocyanin contents were evaluated using generation mean analysis with RCB design for two years. It was found that the inheritance of studied traits did not show an additive-dominance genetic model, indicating that the non-allelic interaction was important for these characters. High broad sense heritability and moderate narrow sense heritability were obtained for anthocyanin content, indicating that this character was controlled mainly by genetic effects, and that both additive and non-additive gene effects were important. In addition, moderate broad sense heritability and lower narrow sense heritability were detected for seed oil content and leaf chlorophyll content, indicating that those two traits were affected by genetic and non-genetic effects, and both the additive and non-additive effects were important. Moreover, the correlations between leaf anthocyanin and chlorophyll contents differed

between generations.

The DArT-seq technique was employed for genotyping through whole genome profiling. The high density genetic linkage map was finally constructed containing 1614 discrete loci with 9212 markers. Based on this map, there were 11, 14 and 8 QTL which controlled the seed oil, leaf anthocyanin and chlorophyll contents respectively, explaining 1.99-18.30%, 9.17-19.13% and 4.45%-13.07% of the phenotypic variation. As well, there was a partially overlapping confidence interval on A06 linkage group for QTL that controlled seed oil and leaf chlorophyll contents. Furthermore, the majority of the QTL were well anchored on the physical map with intervals of 0.02 to 14.54 Mbp. The results were useful for the detection of candidate genes and map-based cloning and the marker-assisted selection of the traits.

School of Crop Production Technology Student's Signature_____

Academic Year 2014 Advisor's Signature_____

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LIST OF ABBREVIATIONS

AC	=	Anthocyanin Content
AFLP	=	Amplified Fragment Length Polymorphism
AIC	=	Akaike Information Criterion
ALA	=	Amino Levulinicacid
BC	=	Back Cross
CC	=	Chlorophyll Content
CIM	=	Composite Interval Mapping
CMS	=	Cytoplasmic Male Sterility
DArT	=	Diversity Arrays Technology
DH	=	Doubled Haploid
EMS	=	Ecotype sensitive Male Sterility
EST	=	Expressed Sequence Tag
GC	=	gametocide
GMS	=	Genetic Male Sterility
HEAR	=	High-Erucic-Acid Rapeseed
HIR	=	High Irradiance Response
IECM	=	Iterated Expectation and Conditional Maximization
LFR	=	Low Fluence Response
MAS	=	Marker-Assisted Selection
ml	=	milliliter

LIST OF ABBREVIATIONS (Continued)

NIRs	=	Near Infrared spectroscopy
PAM	=	Presence / Absence Markers
PH	=	Plant Height
PIC	=	Polymorphism Information Content
QTL	=	Quantitative Trait Loci
RAPD	=	Random Amplified Polymorphic DNA
RE	=	Restriction Enzyme
RFLP	=	Restriction Fragment Length Polymorphisms
RIL	=	Recombinant Inbred Lines
ROS	=	Radical Oxygen Species
SCMR	=	SPAD Chlorophyll Meter Reading
SI	=	Self-Incompatible
SMA	=	Single Marker Analysis
SNP	=	Single Nucleotide Polymorphism
SRAP	=	Sequence Related Amplified Polymorphism
SSR	=	Simple Sequence Repeats
STS	=	Sequence-Tagged Site
w/v	=	weight / volume
w/w	=	weight / weight
µl	=	microliter

CHAPTER I

INTRODUCTION

1.1 Introduction

Rapeseed (*Brassica napus* L., 2n = 4x = 38, genome AACC) is one of the most important member of *Brassicaceae* family and originated as a result of interspecific hybridization between *B. rapa* (AA genome, 2n = 2x = 20) and *B. oleracea* (CC genome, 2n = 2x = 18). Nowadays, rapeseed is the world's third most important source of vegetable oils after palm and soybean and is one of the important sources of biofuel especially in European countries. Thus, the rapeseed breeding strategies are oriented to develop varieties with high and stable seed and oil yield. Since the seed yield and oil content are quantitative traits and their expression are determined by genotype and environmental effects besides genotype by environment interaction (Engqvist et al., 1993, Gunasekera et al., 2006), some basic information such as magnitude and pattern of genetic and heritability of those traits would help to select a suitable breeding procedure. However because the selection of elite genotypes is mostly based on phenotypic values, in many cases the effectiveness of the selection is very low, especially when the phenotypic differences are not under strong genetic control (Marjanović-Jeromela et al., 2011), and the one of the most intractable problems is the interaction between genes and the environment. One approach to solve this complexity is QTL mapping, which is used to identify significant genomic regions associated with quantitative traits based on a molecular linkage map. Furthermore, it is possible to select

the desirable alleles associated with quantitative traits using marker-assisted breeding (Collard et al., 2005) or map-based cloning (Das et al., 2002).

Generally, the oil content of dry seed weight of rapeseed cultivars in China is 38-42% which is lower than that of Australian and European cultivars ranged from 42 to 44% (Li et al., 2007). In the past, breeding for rapeseed was emphasized on reducing the content of glucosinolate and erucic acid , which as a reason leads to the low oil content in China (Liu, 1992). Therefore, better knowledge would be important in helping breeder to control the genetic advance for oil content. Since seed oil in rapeseed is a complex quantitative trait controlled by multiple genes and influenced by environmental factors (Si et al., 2003, Zhao et al., 2005), and varies quantitatively among germplasm of rapeseed due to the complex regulation of multiple genes on metabolism of seed oil (Barker et al., 2007), the genetic control of oil content in rapeseed were reported differently under specific materials. Such as controlled by additive effect (Delourme et al., 2006, Shen et al., 2005, Variath et al., 2009, Zhang et al., 2006), additive-dominant-epistasis genetic model (Wang et al., 2010) and one additive major gene plus additive and dominance polygenes (Zhang et al., 2006). Moreover, the earlier QTL controlling seed oil content were detected to be closely associated with variation of erucic acid content (Ecke et al., 1995). Subsequently, many QTL were identified and most of those QTL can explain less than 20% of phenotypic variation (Wang et al., 2013). In order to compare the QTL detected on different populations, the consensus genetic linkage maps were constructed for detecting the consensus QTL based on the same markers (Jiang et al., 2014, Lombard et al., 2001, Raman et al., 2013).

In addition, leaf anthocyanin and chlorophyll are important yield-related

pigments in plant. The anthocyanin is commonly responsible for orange-red to violet-blue color in plant tissues (Tanaka et al., 2008), and related with the seed yield though the aspect of utilization as morphological marker and resistant to abiotic stresses. Such as breeding for restorer line or temporary maintainer line with purple-red leaf and further application for rapeseed hybrid had been recommended to identify the false hybrid (Wang et al., 2007, Wu et al., 2007). Moreover, rapeseed cultivars with anthocyanin in leaf are classified as drought and frost resistant since the anthocyanin have been proved to be the important function in photosynthetically active tissues of winter rapeseed involved in the protection of mesophyll against cold-induced radiation stress (Solecka et al., 1999), and in ameliorating environmental stresses induced by visible and UV-B radiation, drought and cold temperature (Chalker‐Scott, 1999). Number of Studies indicated that the biosynthesis of anthocyanin was regulated by transcription factors, such as MYC, MYB, WD40-like protein, WRKY, MADS and TFIIIA-like protein (Cichy et al., 2014). In *B. rapa* L. some QTL for seedling anthocyanin (Rahman et al., 2014), or anthocyanin-regulating factor had been identified (Hayashi et al., 2010, Mol et al., 1998). However, the studies on genetic control of anthocyanin mainly focused on the seed color in *B. napus* L., (Jiang et al., 2013, Lu et al., 2012), but now more and more emphasis had been focused on the leaf anthocyanin due to it containing antioxidant (Zhao et al., 2013).

Chlorophyll is the pigment primarily responsible for harvesting light energy used in photosynthesis. Both chlorophyll A and B function to convert light energy to stored chemical energy, thus, chlorophyll content can directly determine photosynthetic potential. Especially, the chlorophyll content in leaf which is one of

the most important factor that determine the photosynthesis capacity. The yield and biomass accumulation of crops directly affected by photosynthetic rate which has a positive correlation with yield potential. The biochemical and genetic approaches were applied to understand the inheritance and biosynthesis of chlorophyll (Oster et al., 2000, Zhao et al., 2014). Nowadays, all the genes for the chlorophyll biosynthetic steps in angiosperm plants have been identified (Beale, 2005), and the whole pathway of chlorophyll biosynthesis can be subdivided into four parts including the formation of 5-aminolevulinic acid, the formation of a pyrrole ring porphobilinogen and the synthesis of the first closed tetrapyrrole, the formation of protoporphyrin IX (Proto IX) and the formation chlorophyll from Proto IX (Tripathy et al., 2012). In *B. campestris* L., some QTL were identified for chlorophyll content, accounting for 7-17% of the phenotypic variation (Ge et al., 2012). In addition, a locus involved in chlorophyll biosynthesis in *B. napus* was mapped to a region of A01 (Zhao et al., 2014).

In this study, we described the gene action for seed oil, leaf anthocyanin and chlorophyll contents, and identified the QTL associated with the traits based on the high-density map which was constructed by DArT-seq markers. The QTL regions also were matched to the physical map. This knowledge may be useful for rapeseed breeding on increasing the oil yield and seed yield-related traits (leaf anthocyanin and chlorophyll contents).

1.2 Significance of the Study

Study on increasing the rapeseed oil and seed yield is a mainly objective on rapeseed breeding due to it directly related to production efficiency. In this study, the leaf anthocyanin and chlorophyll contents were considered to be two important

yield-related pigments in rapeseed. However, the limitation of anthocyanin in most rapeseed line (with green leaf) would be a reason that only a few study on rapeseed anthocyanin. We developed a novel rapeseed inbred line, 10-Zi006, with rich anthocyanin content and would be benefit on improving the resistance to biotic and abiotic stresses. In addition, it is a well material for the inheritance studies of anthocyanin. However, the possible effects of anthocyanin were confounded by a decrease in photochemical efficiency with chlorophyll loss in senescing leaf (Manetas et al., 2011). Moreover, a significant decrease of photosynthetic pigments simultaneous with an increase of anthocyanin was measured in poinsettia bract development (Slatnar et al., 2013). In this way, it is very important to study the genetic control of anthocyanin and chlorophyll contents and their correlation. In addition, the genetic analysis would be useful on breeding for high oil content rapeseed. This study may provide some knowledge on increasing the seed oil content, leaf anthocyanin and chlorophyll contents simultaneously by genetic selection or marker-assistant selection or gene pyramiding.

1.3 Research Objectives

1. To study the inheritance of seed oil, leaf chlorophyll and anthocyanin contents in rapeseed.

2. To construct genetic linkage map and identify QTL associated with seed oil, leaf chlorophyll and anthocyanin contents in rapeseed.

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CHAPTER II

REVIEW OF LITERATURE

2.1 General information of rapeseed (*Brassica napus* L.)

Rapeseed is grown in the temperate areas of the world covering mainly in North America, northern part of Europe, Canada, China and India. It is obtained from the species of *Brassica*, members of Cruciferae. *Brassica* crops consists of six species, of which *B. nigra* (n=8; B genome), *B. oleracea* (n=9; C genome) and *B. campestris* (n=10; A genome) are diploid monogenomic species. The other three, *B. carinata* (n=17), *B. juncea* (n=18) and *B. napus* (n=19; AACC genome) are species which evolved in nature through hybridization between any two of the diploid species. All three genomes are partially homologous; the genetic information in all three genomes is similar, only its organization and distribution on the chromosomes is different. The botanical relationships of these species are illustrated by the "U triangle" (Figure 2.1) which was proposed by a Japanese scientist (U, 1935). *B. napus* L. is an allopolyploid with 19 pairs of chromosomes (n=19), derived from the A and C genomes of *B. campestris* and *B. oleracea*, respectively.

The cultivation of rapeseed started with oleiferous varieties of *B. rapa*. However, the *B. napus* which appeared around the year 1600 has progressively taken the supremacy in this role. Now, rapeseed is the world's third most important source of vegetable oils after palm and soybean, it contributes about 15% of world vegetable oil consumption (http://soystats.com/international-world-vegetable-oil-consumption/). On

the other hand, the rapeseed production has witnessed a steady upward movement and obtained 71.0 million metric tons in 2013, it contributes about 14% of the world vegetable oil production (http://soystats.com/international -world-oilseed-production/). With improvement of rapeseed oil, the low erucic acid varieties enhanced its value as edible oil, particularly among the health conscious consumers and varieties with low glucosinolates increased the value of its defatted meal for use as a feed for livestock. The development of double-low varieties with low glucosinolate and erucic acid content has made rapeseed one of the major plant oil sources at the global level, and now there is a constant tendency to increase its share in production of oilseeds.

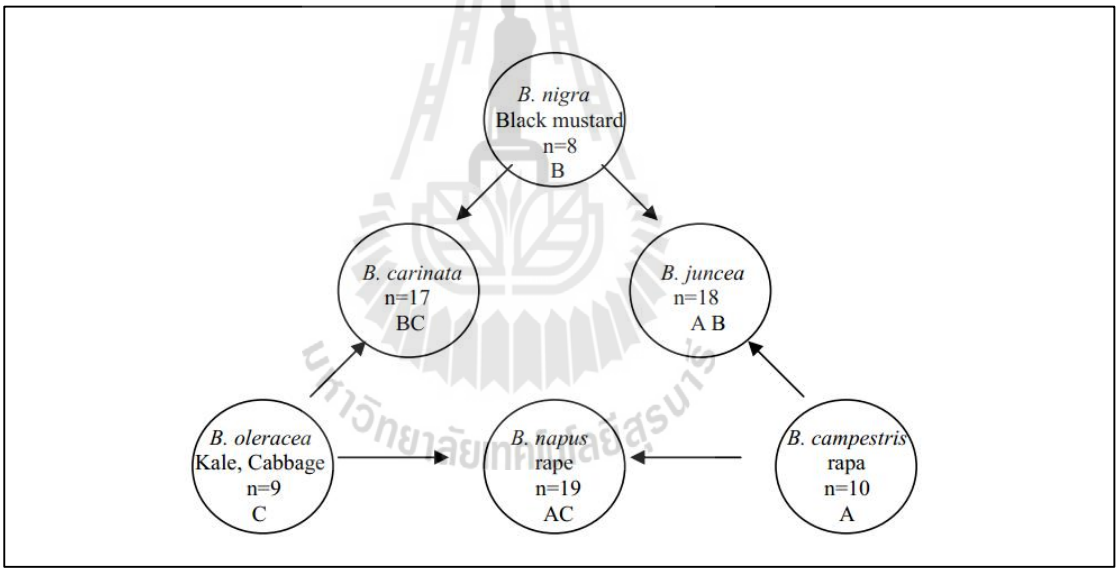

Figure 2.1 Relationships among important *Brassica* species shown by the triangle (U, 1935)

2.2 Rapeseed production in China

China is one of the earliest countries in the world that started to cultivate rapeseed. Its ancient rapeseed cultivation could be traced to pre-historic times, with a

history of 6000-7000 years. And the earliest record of rapeseed cultivation was about 2500 years ago. Since 1987, both China's rapeseed planting area and total yield have been the largest in the world (Wang et al., 2007). China is also the world's largest country in rapeseed consumption. The growing area and total production of rapeseed in China are about 7 million hectares and 11 million tones, respectively (Zhou et al., 2007). Rapeseed production in China is about 43% of the total oil crop yield, just after peanut. The production area is divided into two regions, winter region and spring region. The winter rape is grown in more than 90% of the total rapeseed planting area, while spring rape accounts for only about 10% (Wang et al., 2007). Furthermore, there are three rapeseed species, *B. napus*, *B. rapa* and *B. juncea*, mainly cultivated in China, and the latter two were the dominant rapeseed species in the Chinese long history until *B. napus* was introduced to China in the 1950s. Nowadays, *B. napus* predominates 95%. However *B. rapa*, accounts for 4% approximately and *B. juncea* 1% of China's rape planting area. Since double-low rapeseed and hybrid rapeseed were successfully developed in 1980s and possess many distinguished characters, they are now popularized and have taken more than 70% and 50% of the total rape planting area, respectively (Wang et al., 2007).

2.3 Rapeseed breeding

2.3.1 The objectives of rapeseed breeding and breeding methods

Seed yield, oil yield and early maturity have been the primary objectives of breeding in some countries such as China and India, while the seed quality received greater attention in the European countries, and accordingly the breeding methods also differed. Now, major breeding efforts have been shifted toward the development of

“double-zero” or “double-low” cultivars throughout the globe, and focused on improvement of yield, oil content simultaneously.

The desirable genes within as well as outside the genus are being tapped for the breeding purpose, aided by conventional and biotechnological breeding methods. For this, both interspecific and intergeneric hybridizations have a great potential for creating new variation. Induced mutagenesis has also been useful to create variation for earliness, compact plant type, and yellow seed color, and so on. Conventional breeding methods have received due attention by rapeseed breeders throughout the globe and a large number of varieties have been bred utilizing these methods. Lately, artificial synthesis of amphidiploids and development of herbicide tolerant cultivars has also gained.

Backcrossing has been successfully used to transfer simply inherited traits such as low erucic acid and glucosinolate content into adapted breeding material (Fang et al., 1997). It is also possible to obtain haploid and subsequently doubled-haploid (DH) plants through anther and/or microspore culture. Besides haploid techniques, wide hybridizations using embryo rescue techniques or protoplast fusion can also be used to create novel genetic variation. However, Marker-assisted selection (MAS) has a significant impact on the efficiency of plant breeding routines such as backcrossing programs in case where conventional approaches have not been sufficient. Further improvements can be achieved by genetic engineering.

2.3.2 Breeding for improved productivity

Rapeseed has always been connected with drastic improvements in seed quality. However, study on seed quality does not ignore the importance of high yields. According to the morphological and agronomical characteristics, the yield of rapeseed

consists of the number of siliques per unit area, the number of seeds per silique and the 1000-seed weight (Diepenbrock, 2000). Furthermore, improvement in productivity includes several agronomic parameters, such as early maturation, resistance to lodging and shattering, insects and, particularly, to the major diseases.

2.3.3 Hybrid breeding

The hybrid breeding of rapeseed developed faster in the past few years. The use of male sterility system is one of the means to develop hybrids. Rapeseed is a partially autogamous crop. Thus, the first step for breeding hybrid rapeseed is to develop male sterile line. Rapeseed hybrids tend to have higher yield stability and better adaptation to low input cropping systems than conventional cultivars (Gehringer et al., 2007), which is the reasons that the breeders interest in hybrid varieties. Numerous cytoplasmic male sterility (CMS) systems have been discovered and are used in crop *Brassica*s and two spontaneous male sterile cytoplasms, *nap* and *pol*, are found in *B. napus* (Gustafson et al., 2009). Most other *B. napus* CMS systems result from interspecific or intergeneric crosses, such as *Ogu* CMS was discovered by Japanese scientists as a male sterile line in radish and then was transferred into rapeseed by French researchers (Ogura, 1968). *Ogu* CMS has a very stable sterility however restorer genes had not been found in rapeseed. Subsequently, many other types of male sterility including CMS, genetic male sterility (GMS), ecotype sensitive male sterility or environment sensitive male sterility (EMS), gametocide (GC) and self-incompatible (SI) system were developed and used in production of hybrids (Yu et al., 2007). Many hybrid varieties were developed by using CMS including Pol CMS (Chen et al., 2010, Wang et al., 2012), GMS (Chen, H. et al., 2011, Chen, S. Y. et al., 2011, Qin et al., 2010) in China. These varieties had played very important roles in

increasing rapeseed yield in China. Now hybrid breeding is the major method for development of rapeseed cultivars.

2.4 Rapeseed oil content

2.4.1 Importance of rapeseed oil

Rapeseed (*B. napus* L.) is one of the most important edible oilseed crops in the world, as well as a major potential source of bio-diesel production in Europe (Wang, 2005). It also has wide use of industry. In the crushing industry, about 80% of value of rapeseed is linked to its seed oil content. One percent increase of oil content in rapeseed is equivalent to a 2.3-2.5 % increase in the seed yield (Wang, 2004), so high oil content can significantly improve the efficiency of rapeseed production.

The oil of rapeseed varieties is highly appreciated by people because of its fatty acid profile, which need to reduce total dietary saturated fat intake for health recommendations. The current interest in the nutritional and health effects of fatty acids relates to high intake of saturated fat in the diet with increased levels of blood cholesterol, arteriosclerosis and high coronary heart disease risk (Grundy et al., 1990, Gurr, 1992). However, low erucic acid content rapeseed contains the lowest level (ca. 6 to 7%) of saturated fatty acids with 62 to 65% of its total fatty acids as oleic acid and contains 8 to 10% of linolenic acid (Hu, 2007). Therefore, rapeseed oil with low erucic acid is an important source of monounsaturated fatty acid.

High oleic content and low linolenic content oils are being developed primarily to reduce trans fatty acids formed during the hydrogenation of vegetable oils. Nutritional research suggests that these stereo-isomers of cis-fatty acids may have negative nutritional effects. Trans fatty acids appear to increase serum low-density

lipoprotein (LDL) cholesterol levels and may reduce serum high-density lipoprotein (HDL) cholesterol levels to a greater extent than saturated fatty acids (Mensink et al., 1993). These concerns led to a recommendation to reduce their amount in the diet. Therefore, rapeseed oils with a high oleic acid and low linolenic acid content are good for people health, and it also marketed for bottled salad oil and salad dressings as well as for food applications requiring high cooking and frying temperature stability, including extended shelf-life products. On the other hand, rapeseed oil exhibits a higher content of essential fatty acids, especially α-linolenic acid, and is the only plant oil with an optimal 2:1 proportion of linoleic to α-linolenic acid.

Rapeseed oil also has many potential uses other than as oil for nutrition (Piazza et al., 2001). Historically, rapeseed oil was used mainly for industry and for domestic lighting. The use of rapeseed oil as a lubricant or additive to petroleum-based lubricants was developed after the replacement of steam power by diesel engines, because rapeseed oil clings to water-treated metal surfaces better than other lubricants. Besides being used industrially in many applications in which almost any vegetable oil can be used, rapeseed oil with high content of erucic acid in particular has considerable advantages in specific applications due to the properties of this long-chain fatty acid. High-erucic-acid rapeseed (HEAR) oil contains ca. 50% erucic acid. The special properties of HEAR oil include high smoke and flash points, stability at high temperatures, durability and the ability to remain fluid at low temperatures. The principal end use of HEAR is to produce erucamide, which is used as a slip additive in polyethylene and polypropylene manufacture to reduce surface friction and prevent adhesion between film surfaces. Erucamide is a relatively large complex molecule, making it difficult and expensive to produce synthetically from

petrochemicals. HEAR oil is also used in printing inks and lubricants and has a range of other applications (Piazza et al., 2001).

Nowadays, low erucic acid content rapeseed oil is the primary feedstock of commercial biodiesel production in Europe (Wang, 2005). The driving forces behind the use of biodiesel fuels are mainly environmental and energy concerns. In general, vegetable oil biodiesel fuels, being simple alkyl esters, have the following advantages over diesel fuel: as a neat fuel or in blends with diesel fuel, they produce less smoke and particulates, have higher octane values, produce lower carbon monoxide and hydrocarbon emissions, and are biodegradable and non-toxic.

So, it is very important to understand the inheritance and how to increase the oil content of rapeseed since it is as nutritional oil for people's health and wide industrial use.

2.4.2 Inheritance of oil content

It is commonly considered that oil content in *B. napus* is a complex quantitative trait controlled by multiple genes and is influenced by various environmental factors (Pai et al., 1991, Si et al., 2003). Previous studies in oilseed rape (*B. napus*) have indicated that additive gene action is the main genetic factor in the control of seed oil content with dominance and epistasis being not significant (Delourme et al., 2006, Zhao et al., 2006, Zhao et al., 2005). Some reports showed that the inheritance of oil content could be explained by an additive-dominant-epistasis model with dominant and additive effects being the main components. It was found that the oil content of F_1 hybrid seeds in rapeseed was mainly controlled by the maternal genotype and had a significant cytoplasmic effect, while xenia effects had also a certain impact on the oil content of hybrid seeds (Wang

et al., 2010). But other results indicated that oil content was thought to be controlled by only maternal genotypes (Gan et al., 1997), or embryo genetic effects, cytoplasmic effects, maternal genotypes as well as environment interaction effects (Wu, J. et al., 2006, Wu, J. G. et al., 2006).

However, accumulation of seed oil is influenced by environmental conditions, which makes its genetic control complicated and difficult to understand. The oil content of rapeseed with the same genotype had a significant difference varied across growing environments (Zhang et al., 2006). Two pathways might exist in oil synthesis, which are affected by environment. In the first pathway, different members of the gene family involved in the metabolic network are induced by different environmental conditions, of which a few are expressed under normal condition and some others are expressed under stress conditions. The different abilities of these gene members result in variation of oil content and low-altitude environment is preferred to develop stable rapeseed varieties with high oil content (Li et al., 2011).

Using molecular markers, the quantitative trait loci (QTL) for oil content were identified on different chromosomes in *B. napus* (Ecke et al., 1995), which is consistent with the polygenic determinism of the trait (Burns et al., 2003, Delourme et al., 2006, Ecke et al., 1995, Qiu et al., 2006, Si et al., 2003, Zhao et al., 2006, Zhao et al., 2005). Each of the QTL accounted for less than 10% of the total oil content variance (Delourme et al., 2006, Qiu et al., 2006). Some of these QTL coincided with loci controlling erucic acid content, suggesting that it is a major determinant for oil content in oilseed rape (Burns et al., 2003, Ecke et al., 1995, Qiu et al., 2006). Additive effects were shown to be the main factors controlling oil content, with individual additive effect of the different alleles ranging from 0.2 to 1.2% (Delourme

et al., 2006, Qiu et al., 2006). In addition, strong environmental effects underlie variations in oil content (Si et al., 2003, Zhao et al., 2005).

2.5 Anthocyanin

Anthocyanin are to be found in the vacuoles of almost every cell in the epidermal, ground, and vascular tissues of all vegetative organs. They occur not only in leaf but also in roots, hypocotyls, coleoptiles, stems, tubers, rhizomes, stolons, bulbs, corms, phylloclades, and axillary buds. It is present in some bryophytes, ferns, and conifers, and are virtually ubiquitous in angiosperms (Lee et al., 2002). Anthocyanin also are flavonoid pigments which most commonly responsible for red, purple, and blue coloration in plant tissues. The functions for cellular anthocyanin have been made significant progress (Chalker-Scott, 2002, Chalker‐Scott, 1999, Close et al., 2003, Gould, K. et al., 2002, Gould, 2004, Hoch et al., 2001, Lee et al., 2002, Manetas, 2006, Steyn et al., 2002, Stintzing et al., 2004). It was shown that anthocyanin have key functions including protection of chloroplasts from the adverse effects of excess light, attenuation of UV-B radiation, and antioxidant activity. Anthocyanin have also been suggested to have a more indirect role, as modulators of reactive oxygen signaling cascades involved in plant growth and development, responses to stress, and gene expression.

2.5.1 Physiological function of anthocyanin in plant tissues

Anthocyanin in leaf most occur as vacuolar solutions in epidermal and/or mesophyll cells (Hooijmaijers et al., 2007, Kunz et al., 1993, Lee et al., 2001). It serves as photo protection by shielding photosynthetic cells from adverse effects of strong light. The anthocyanin in leaf absorb more light in the green and yellow

wavebands (Gitelson et al., 2001). The energy of absorbed quanta is not transferred to the chloroplasts. Indeed, the chlorenchyma of red leaf may receive considerably less green light than do those of structurally comparable green leaf (Gould, K. et al., 2002), and red leaf may develop the morphological and physiological attributes of shade leaf (Manetas, 2006). These because of light-energy absorbed by anthocyanin is not used for photosynthesis, but is rather dissipated as heat. The optical properties of anthocyanin are therefore of functional importance, as they directly influence the wavelengths and intensity of light available to be used for photosynthesis within a leaf. Considering that blue-green light contains the most highly-abundant, high-energy wavelengths of the solar spectrum, that blue-green light deeply penetrates leaf tissues (Cui et al., 1991), and that wavelengths responsible for photoinhibition and photo-bleaching are primarily between 520 and 680 nm (Manetas et al., 2003), the value of blue-green light absorbing pigments during high sunlight exposure becomes increasingly apparent. The down-regulation of internal light for photoprotection has thus become a popular explanation for the widespread occurrence of anthocyanin pigments in leaf and other photosynthetic tissues, especially in high-light habitats.

In addition to their capacity to protect plant tissues from excess visible radiation, anthocyanin have also been implicated in the protection from ultraviolet radiation. To fortify themselves against the harmful effects of UV radiation, plants have developed multifarious mechanisms to diminish UV penetration into plant tissues, including the synthesis of UV-absorbing phenolic compounds (Ryan et al., 2005). The biosynthesis of anthocyanin and other flavonoids is known to be activated in many plant species by UV exposure (Mendez et al., 1999, Singh et al., 1999, Takahashi et al., 1991). Most anthocyanin, especially those that are acylated, can

absorb biologically-active UV radiation (Giusti et al., 1999), and it has been suggested that their function in vegetative organs may be to buffer tissues against UV damage by attenuating the excess energy (Kaliamoorthy et al., 1994, Li et al., 1993, Takahashi et al., 1991).

On the other hand, anthocyanin have also been implicated as functioning as an in vivo antioxidant. However, there has been some debate as to how effective a vacuolar antioxidant could be in protecting tissues against radical oxygen species (ROS) generated in the chloroplast (Manetas, 2006). It has been shown that H_2O_2 is capable of penetrating both chloroplast and vacuolar membranes, and that anthocyanin can effectively neutralize these ROS in cells proximate to high light stress (Gould, K. S. et al., 2002, Kytridis et al., 2006, Nagata et al., 2003, Yamasaki et al., 1996).

Anthocyanin, as a solute, also can decrease leaf osmotic potential serving as osmotic adjustment and maintenance of turgor pressure in the presence of drought stress during senescence (Chalker‐Scott, 1999). In general, anthocyanin synthesis is inducible under conditions which promote osmotic stress, including high salinity (Kaliamoorthy et al., 1994, Ramanjulu et al., 1993), drought, and high sugar treatment (Sakamoto et al., 1994, Suzuki, 1995, Tholakalabavi et al., 1997) . Furthermore, species with high anthocyanin content seem to be common in environments characterized by low soil moisture (Schemske et al., 2001), and appear to be more tolerant of drought conditions (Beeson Jr, 1992, Paine et al., 1992). Considering role of anthocyanin under water stress, it may be directly involved in osmotic regulation, preventing transpirational water loss while helping maintain turgor pressure (Chalker-Scott, 2002, Chalker‐Scott, 1999). However, anthocyanin content in leaf cells is generally much too low to sufficiently support an osmoregulatory function,

and may just contribute less than 1% to the osmotic potential of a leaf (Manetas, 2006). Furthermore, soluble sugars and K^+ are ubiquitous among plants while anthocyanin are not, and are known to be the primary contributors to osmotic adjustment in several species. Another explanation for the association between anthocyanin and water stress is that anthocyanin are synthesized to alleviate oxidative stress resulting from low water potentials, either through acting as an antioxidant, or in photoprotection. Under this latter scenario, anthocyanin would be inducible by water stress, but may not function directly in its alleviation.

On the other hand, anthocyanin may function to warm leaf and increase rates of transpiration and metabolism, or protect against cold temperatures since it increase energy absorption by the leaf, and convert light energy into heat. This would be most effective in colder climates, as leaf energy balance models dictate that leaf may be more easily uncoupled from air temperature when air temperature is low (Campbell et al., 1998). Indeed, some evidence exists for a warming function of anthocyanin in other plant tissues at high altitudes (Sturgeon et al., 1980).

2.5.2 The synthesis of anthocyanin

Anthocyanin are secondary metabolites derived from the precursors that are malonyl-CoA and p-coumaroyl-CoA (Holton et al., 1995). Light is the main factor that effects anthocyanin gene expression, the intensity of light is also directly proportional to anthocyanin concentration (Hughes et al., 2005). Anthocyanin production is significant under low-fluence conditions, but generally low in quantity, and shows the general characteristics of a low fluence response (LFR) of phytochrome B. However, it may be induced in greater quantities under prolonged exposure to high irradiance as a high-irradiance response (HIR). This process shows peaks in the action

spectra not only in the red, but also UV-A, UV-B and blue wavelengths, indicating involvement of phytochrome A (activated by red light), cryptochrome (blue and UV-A), and another UV-B receptor. Under high light, phytochrome and cryptochrome are synergistic, and anthocyanin synthesis under exposure to red plus blue/UV-A is greater than the sum of anthocyanin production under red light alone and blue/UV-A light alone. Once these sensory pigments are activated, they themselves activate (i.e. phosphorylate or reduce) transcription factors involved in expression of the anthocyanin structural genes.

However, anthocyanin synthesis is relating to certain plants growth conditions, usually those associated with abiotic stress. Heat/cold, drought, osmotic, and nutrient stress are known to induce anthocyanin synthesis in many species under high light (Chalker‐Scott, 1999), the coming of certain ontogenetic stages or seasons which render plants vulnerable to these stresses. The additional regulatory mechanisms are in place to control anthocyanin synthesis except those which are light-mediated. Hydrogen peroxide (H_2O_2) is known to function as a regulatory molecule in anthocyanin synthesis (Vanderauwera et al., 2005). Indeed, many of the conditions described above also correspond with elevated production of radical oxygen species (ROS) and hydrogen peroxide.

Similarly, sugars also play a role in stress signaling in plant systems and elevated levels of reducing sugars are known to induce anthocyanin synthesis (Hughes et al., 2005, Teng et al., 2005, Weiss, 2001); In a general, stressful conditions may correspond with those that have high sugar concentrations and/or elevated production of H_2O_2, which may act as a natural signal inducing anthocyanin gene expression (Hughes et al., 2005, Teng et al., 2005).

Synthesis of anthocyanin occurs in the cytoplasm until the precursor anthocyanidin. Up to 19 different naturally-occurring anthocyanidins have been reported in plant tissues (Iwashina, 2000), though the bronze-colored cyanidin-3-glucoside is the most common form encountered in leaf (Lee et al., 2002). In the cytoplasm, anthocyanidin is tagged with a glutathione by glutathione-S transferase, and is transported via glutathione pump into the vacuole (Marrs et al., 1995). It is within the vacuole that anthocyanin take on their species-specific coloration, which results from hydroxylation, methylation, sugar addition, or acylation.

2.6 Chlorophyll

Chlorophyll is the pigment primarily responsible for harvesting light energy used in photosynthesis. Chlorophyll was found in cyanobacteria and the chloroplasts of algae and plants in which the molecule consists of a porphyrin head and a long hydrocarbon. There are four types of chlorophyll designated as chlorophyll a, b, c, and d. Chlorophyll a and b both are important photosynthetic pigment in higher plants. Chlorophyll c is found in the diatoms, dinoflagellates, and brown algae. However, chlorophyll d is found only in the red algae.

2.6.1 Chlorophyll and photosynthesis

Chlorophyll allows plants to absorb energy from light. Chlorophyll molecules are specifically arranged in and around photosystems that are embedded in the thylakoid membranes of chloroplasts (Finzi et al., 1989). The function of the vast majority of chlorophyll is to absorb light and transfer the light energy by resonance energy to a specific chlorophyll pair in the reaction center of the photosystems. The

function of the reaction center chlorophyll is to use the energy absorbed from the other chlorophyll pigments in the photosystems to undergo a charge separation, a specific redox reaction in which the chlorophyll donates an electron into electron transport chain (Trlbutsch, 1972). The charged reaction center chlorophyll ($P680^+$) is then reduced back to its ground state by accepting an electron. In Photosystem II, the electron that reduces $P680^+$ ultimately comes from the oxidation of water into O_2 and H^+ through several intermediates (Schatz et al., 1988). This reaction is how photosynthetic organisms such as plants produce O_2 gas. Photosystem I typically works in series with Photosystem II; thus the $P700^+$ of Photosystem I is usually reduced, via many intermediates in the thylakoid membrane, by electrons ultimately from Photosystem II.

The electron flow produced by the reaction center chlorophyll pigments is used to shuttle H^+ ions across the thylakoid membrane, setting up a chemiosmotic potential used to produce ATP chemical energy, and those electrons ultimately reduce $NADP^+$ to NADPH, a universal reductant used to reduce CO_2 into sugars as well as for other biosynthetic reductions. Although the reaction center chlorophyll-protein complexes can directly absorb light and perform charge separation without other chlorophyll pigments, but the absorption cross section is small. Thus, the remaining chlorophyll in the photosystem and antenna pigment protein complexes associated with the photosystems all cooperatively absorb and funnel light energy to the reaction center.

2.6.2 The function of chlorophyll in plant

Both chlorophyll a and chlorophyll b function as conversion of light energy for storing chemical energy, thus, chlorophyll content can directly determine

photosynthetic potential and primary production (Curran et al., 1990, Pružinská et al., 2003, Richardson et al., 2002). In cereal crops, such as barley, the chlorophyll content and photosynthesis capacity have been considered to be the important determinants of grain yield (Xue et al., 2008). However, the chlorophyll content in leaf is one of the most important factor that determine the photosynthesis capacity. The yield and biomass accumulation of crops directly affected by photosynthetic rate which has a positive correlation relationship between leaf photosynthetic rate and the yield potential (Jiang et al., 2002, Ohsumi et al., 2007)

In addition, chlorophyll gives an indirect estimation of the nutrient status because much of leaf nitrogen is incorporated in chlorophyll (Steele et al., 2008, Xue et al., 2009). Furthermore, leaf chlorophyll content is closely related to plant stress and senescence (Pružinská et al., 2003, Steele et al., 2008).

2.6.3 Chlorophyll biosynthesis

In plants, chlorophyll is synthesized from succinyl-CoA and glycine (Rebeiz et al., 1973). However, the protochlorophyllide is the immediate precursor of chlorophyll a and b. In Angiosperm plants, the conversion of protochlorophyllide to chlorophyll is light-dependent and such plants are pale (etiolated) if grown in the darkness (Adamson et al., 2006). Green algae differently have an additional light-independent enzyme and grow green in the darkness instead (Reinbothe et al., 2010).

Chlorophyll itself is bound to proteins and can transfer the absorbed energy in the required direction. Protochlorophyllide occurs mostly in the free form and under light conditions, acts as a photosensitizer, forming highly toxic free radicals. Hence, plants need an efficient mechanism of regulating the amount of chlorophyll precursor.

In angiosperms, this is done at the step of amino levulinicacid (ALA), one of the intermediate compounds in the biosynthesis pathway (Rebeiz et al., 1973). Plants that are fed by ALA accumulate high and toxic levels of protochlorophyllide (Meskauskiene et al., 2001).

2.7 Study on gene action of rapeseed

It is important for improvement of crop interesting traits that researcher get information on inheritance and gene action by designing efficient methods. Generation mean analysis commonly used for study on inheritance of certain traits in crop, in which additive, dominance, and epistatic effects can be separated using inheritance model (Bai et al., 2000, Meena et al., 2010), and various gene effects are equated to means of F_1, F_2, backcrosses and other generations derived from two inbred lines. Inheritance of bacterial spot disease in *Capsicum annuum* L. was conducted using the similar method (Riva et al., 2004). To study the genetic basis of tolerance to flooding in maize, two maize lines were used as parents to form F_1, F_2, BC_1 and BC_2 generations. The results showed that tolerance to flooding is controlled by many genes with complementary effect (e Silva et al., 2007). The method using generation mean for inheritance analysis of interesting characters has been widely used in rapeseed, characters such as earliness, height, and leaf number (Ringdahl et al., 1986); quality characters such as erucic acid content and oleic acid content (Qi et al., 2001); resistance to disease as white rust resistance in *B. juncea* (Kumar et al., 2007).

2.8 Molecular mapping of quantitative trait loci

The identification of genes associated with quantitative traits is complicated by

the segregation of multiple genes that often produce minor effects. These traits are difficult to study because the phenotype does not easily provide an insight into the genotype, unlike most simple single-gene traits with major effects (Kearsey et al., 1998). Environmental effects further confound analysis by contributing to the phenotypic expression in both additive and non-additive effects. In general, it is not always possible to determine if a chromosomal region contributing to a quantitative trait is due to one or more linked genes unless the heritability of the trait is high or extremely large population sizes are employed (Tanksley, 1993).

The term Quantitative Trait Loci (QTL) is used to describe regions of the chromosome contributing to the variability of traits of interest (Liu, 1997). The identification of QTL require a segregating population structure, a phenotypic trait or traits that have been measured in one or more environments, polymorphic molecular markers, and a genetic linkage map. Segregating populations are generated from crosses between parents (commonly inbred line) that are thought to possess allelic differences at genes influencing or regulating the traits of interest. Crosses are generally made between parents that differ significantly in some phenotypic measurement, or may be made between two or more high scoring parents as long as some measure of significant genetic variability is present (Dudley, 1997).

2.8.1 Mapping populations

The F_2 population, back cross population (BC), doubled haploid (DH) population, and recombinant inbred lines (RI) are four types of population that commonly used for map construction. The F_2 population can be easily and quickly developed and contains all possible combinations of parental alleles. BC population is widely used for genetic mapping. However, the BC population contains less genetic

information than the F_2, which results in additive effects cannot be distinguished from dominance effects, and some types of epistatic effects are also confounded. A major disadvantage of both F_2 and BC populations is that the genotype variation in individuals and cannot repeat experiment in different locations, which would be expensive to take more genotyping for QTL mapping. The individuals in DH population are completely homozygous. For this reason, the DH population can produce genetically identical progeny by self-pollination which can provide replicated testing for phenotypes in different environments. However, it is impossible to estimate dominance effect and epistasis effects; secondly. RIL population can be made by traditional means by selfing several generations starting from F_2 by the single seed descent approach until almost all of the segregating loci become homozygous. Theoretically, the genetic structure of a RIL population is identical to DH population. However genetic distances based on RIL population are enlarged since the many generations of selfing will increase the chance of recombination. A major disadvantage for RIL population is similar as for DH population. Furthermore, development of RIL population takes long time and it is impossible for all individuals to be homozygous at all segregating loci through limited generations of selfing, which decreases the efficiency for QTL mapping.

2.8.2 Genetic markers

A genetic marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. Generally, DNA markers can be classified into hybridization-based marker such as RFLP (Botstein et al., 1980), EST (Qin et al., 2001), and PCR-based marker such as AFLP (Vos et al., 1995), SSR, RAPD (Williams et al., 1990), STS (Inoue et al., 1994). DArT is a

cost-effective genotyping technology detecting all types of DNA variation (SNP, indel, CNV, methylation). The DArT technique was invented to overcome some of the limitations of other molecular marker technologies such as RFLP, AFLP and SSR (Kilian et al., 2012). The core of DArT technology is a genome complexity reduction and this methods provide a significant advantage via an intelligent selection of genome fraction corresponding predominantly to active genes (http://www.diversityarrays.com). This selection is achieved through the use of a combination of restriction enzymes which separate low copy sequences from the repetitive fraction of the genome. For Brassica, the technology was optimized by selecting the PstI- MseI method to reduce the AnAnCnCn genomic complexity and produced SNP and Presence/Absence Markers (PAM) markers (Raman et al., 2014).

The DArT technique had been employed widely on genetic linkage map construction for varied crops through the whole-genome profiling (Iorizzo et al., 2014, Milczarski et al., 2011, Schouten et al., 2012, Tomaszewski et al., 2012). In addition, the high-density composite or consensus map had been constructed using DArT marker for genetic studies, such as segregation distortion analysis (Alheit et al., 2011, Li et al., 2010); Pan-genomic evolutionary studies (James et al., 2008); QTL identification (Cichy et al., 2014, Cui et al., 2014, Rahman et al., 2014). Moreover, the DArT marker had been used to develop the first genetic map or first complete genetic map in some crops (Oliver et al., 2011, Yang et al., 2011). DArT technique as a high-throughput genotyping platforms was utilized for high-density genetic map construction in rapeseed (Raman et al., 2014). Furthermore, the first *B. napus* consensus map was constructed based on the Diversity Arrays Technology (DArT) and non-DArT markers, this consensus map can be used to identify candidate genes

underlying natural genetic variation for traits of interest (Raman et al., 2013).

2.8.3 Construction of genetic linkage maps and QTL analysis

A deviation from the expected frequencies of any two markers suggests linkage and provides the basis for the development of genetic linkage maps. So, the tests of independence need to be done either through chi-square or maximum likelihood methodologies by observing the recombination frequencies between pairs of molecular markers and making comparisons to expected frequencies. Recombination frequency is the probability that a gamete produced by a heterozygous individual is a recombinant in regard to two given markers or genes. Recombination frequencies vary from 0 to 0.5 and do not directly correspond to physical distance as regions in the genome vary in their rate of recombination. We can get the mapping distance which is calculated from recombination frequency by use of mapping functions. The unit of distance is Morgans, which is defined as the physical distance on a chromosome where at least one recombination event is expected to occur during meiosis. The most common mapping functions are Haldane's that allows for double recombinants among linked markers with no interference, and Kosambi that factors in interference among loosely linked markers. With tight linkage (<10 cM) between markers both functions produce similar results. Numerous computer programs such as Mapmaker (Lander et al., 1987) and JoinMap (Stam, 1993) have been developed to analyze polymorphic marker data and construct genetic linkage maps. Those programs calculate recombination frequencies among markers, assign markers to linkage groups, and produce LOD scores to allow comparisons with an alternative hypothesis. Deviations from expected Mendelian segregation patterns by reducing recombination can result in non-representative genetic distances and linkage relationships. This is

particularly a problem in widely divergent or interspecific crosses (Rick, 1972).

Since the first genetic map for *B. napus* L. was constructed by using RFLPs marker (Landry et al., 1991), study on construction of molecular genetic map is becoming a research hotspots and many genetic map were constructed by researchers (Cheung et al., 1997, Ferreira et al., 1994, Parkin et al., 1995, Piquemal et al., 2005, Saal et al., 2001, Sharpe et al., 1995). Nowadays, high density genetic maps are considered to facilitate many biological studies including map-based cloning, association genetics and marker assisted breeding. However, the selection of computer programs, as well as genotyping error removal is very important on high-density map construction based upon the high-throughput genotyping platforms. Some available computer programs for linkage mapping are incapable of handling data sets of several thousands of markers and result in prohibitively long calculation times. On the other hand, even small frequencies of scoring error result in high rates of ordering ambiguities between markers within short genetic distances (Van Os et al., 2006). The program SMOOTH combined with JionMap v4 can remove genotyping error. SMOOTH is a tool for genotyping error removal through calculation of the probability of a data point being a ‘‘singleton’’ on the basis of neighboring marker information. The observation of singletons depends on their context of flanking markers. Therefore, singletons are removed in an iterative process, singleton removal, reordering of markers, singleton removal, reordering, etc., thereby gradually relaxing the statistical threshold of singleton identification (van Os et al., 2005). So the SMOOTH always been combined with any of the mapping programs, such as RECORD-WIN; JionMap and MST_{MAP}, etc. Even the loss of a few percentages of the data though the genotyping error removal, however it is obviously less damaging to

the map than having similar levels of genotyping errors (van Os et al., 2005). For high-density map construction, a novel online program named MST_{MAP} was used to cluster and order thousands of markers. This method is computationally very efficient and also gracefully handles missing observations and is capable of tolerating some genotyping errors. MST_{MAP} is freely available in the public domain at http://www.cs.ucr.edu/yonghui/MST_{MAP}.html (Wu et al., 2008).

A number of statistical approaches based upon conditional probabilities of expected QTL genotypes, as inferred by marker phenotypes, have been used to identify relationships among markers and genes associated with quantitative traits. These techniques utilize either the difference between marker class means (the linear model) or use information from the entire marker trait distribution (maximum likelihood). Maximum likelihood is generally the more statistically rigorous approach, however, it has been demonstrated that maximum likelihood estimates reduce to least square estimates when data is normally distributed. Linear model methodologies can be written to existing computer programs, are less computationally exhaustive and are more accessible to fitting new parameters (Haley et al., 1992). The linear model can utilize t-tests, ANOVAs or regression to detect significant differences among marker class means.

Single factor analysis tests the significance at each marker loci throughout the genome, however, the effect of the putative QTL and the recombination fraction between the QTL and the marker are confounded. Only if the QTL and the marker co-segregate, the analysis will produce an unbiased estimate of QTL effect, otherwise it will be underestimated by a factor of 1-2 r (where r is the recombination fraction between marker and QTL). It has been suggested that it is reasonable to use single

factor analysis for initial detection of general QTL location but that further analysis should be conducted to estimate position and magnitude of effect.

Mapping using flanking markers compensates for many of the limitations of single factor analysis (Lander et al., 1989). Interval analysis using flanking markers allows for better resolution of putative QTL by resolving the confounding effects of recombination and magnitude. Interval mapping tests for QTL presence at and between adjacent markers at predetermined increments (i.e. 1 or 2 cM) and produces likelihood score for each position. QTL are declared at the point the likelihood score reaches its maximum and exceeds a predetermined level. Traditionally, LOD scores between 2.5 and 3.0 have been used to declare putative QTL. Regression models have also been adapted to approximate maximum likelihood methodologies and based upon regression of phenotypes on the conditional probabilities of QTL genotypes as inferred by the flanking markers (Haley et al., 1992). Composite interval analysis increases the power of interval mapping by fitting regression coefficients for selected markers to account for variance arising from regions other than the target interval (Zeng, 1994).

A number of methodologies have been proposed for determining the appropriate significance levels for declaring putative QTL linked to a given marker. The type Ⅰ error (a) is the probability of declaring a false positive (i.e. declaring a QTL linked to a marker when in fact, there is no linkage). If the type Ⅰ error is set at a 0.05 probability level, for example, when 100 independent tests are performed, it is expected on average that five times tests out of 100 will declare linkage between a QTL and a marker when in fact no linkage is present (Dudley, 1993). It has been proposed that significance levels be set equivalent to 0.001. However, using such

stringent levels can increase proportionately the probability of incurring a type II error (i.e. the failure to detect a linked QTL when one is present). It has become common practice to use LOD levels of 3.0 to provide a relatively stringent test of significance while still allowing for the detection of QTL. The power of an experiment, that is the probability correctly rejecting the null hypothesis (no linked QTL), is affected by the population size (number of individuals in each marker class), the magnitude of effect to be detected, and the marker spacing throughout the genome (Darvasi et al., 1993).

2.8.4 Identification of QTL in *Brassica napus* L.

Yield is the most important and complex trait for genetic improvement of rapeseed. Eighty five quantitative trait loci (QTL) for seed yield along with 785 QTL for eight yield-associated traits were identified from 10 natural environments, 330 consensuses QTL were clustered and integrated into ill pleiotropic unique QTL by meta-analysis. Of which, 47 QTL were relevant for seed yield. The complexity of the genetic architecture of yield was demonstrated (Jiaqin et al., 2009). Twenty-nine QTL were common detected for yield and yield related to traits which include plant height (PH), height of lowest primary effective branch (HPB), length of main inflorescence (LMI), silique length (SL), number of primary branches (FB) and silique density (SD) between the two populations (Wei et al., 2007). Furthermore, different kinds of mode of inheritance of QTL (additive; partial-dominant; full-dominant; over-dominant) and epistatic interactions (additive by additive; additive by dominant/dominant by additive; dominant by dominant) were observed and epistasis, especially additive by additive epistasis, seemed to be the major genetic basis of heterosis in rapeseed (Jiaqin et al., 2011). The QTLs detected for yield also were suitable to predict hybrid performance and heterosis in *B. napus* (Jin-Xiong et al., 2006). In order to advance research on

seed-yield and yield-related traits in *B. napus*, a comparative mapping between *O. sativa* and *B. napus* was conducted. Results showed that a total of 37 *O. sativa* and *B. napus* homologues were located in similar yield-related QTL between species, which may be helpful for agronomic gene characterization in *B. napus* based on what is known in *O. sativa* (Li et al., 2012). It is a novel approach to improve seed yield of hybrid spring canola by introgression of winter germplasm into spring canola (*B. napus* L.). QTLs for seed yield and other traits were genetically mapped to determine the effects of genomic regions introgressed from winter germplasm into spring canola. Six QTL were detected for the winter alleles increased seed yield. One of these QTL explained 11 and 19% of the phenotypic variation in the two Canadian environments. However, the winter allele for another QTL that increased seed yield was linked in coupling to a QTL allele for high glucosinolate content, suggesting that the transition of rapeseed into canola could have resulted in the loss of favorable seed yield alleles (Quijada et al., 2006).

Increasing the oil content is another important objective in rapeseed breeding. So, to get better knowledge on the genetic determinism of seed oil content is a method to increase the oil content. It was demonstrated that additive effects are the main factors contributing to variation in oil content by mapping QTL in two populations. Some of the QTLs associate with oil content were more consistently revealed across different genetic backgrounds. However some of the QTLs were specific to one genetic background with potentially some original alleles (Delourme et al., 2006). Using a recombinant doubled haploid (DH) as mapping population, 27 QTL were identified on 14 linkage groups, individual QTL for oil content explained 4.20–30.20% of the total phenotypic variance (Gang et al., 2010). Oil content in

rapeseed is generally negatively correlated with protein content and influenced by plant developmental and yield related traits. It is interesting that two QTLs were identified in a doubled haploid population, which had control oil content independent from protein content and which could be used in practical breeding programs to increase oil content without affecting seed protein content (Jianyi et al., 2006). Other QTLs were identified in the location with different latitude indicate that oil content was significantly correlated with growing environment (Li et al., 2011).

On the other hand, improvement of seed quality was focused on fatty acid balance and low seed glucosinolate content and erucic acid content (Nesi et al., 2008). QTLs linked to palmitic acid (C16:0) content, stearic acid (C18:0) content, oleic acid (C18:1) content, linoleic acid (C18:2) content, linolenic acid (C18:3) content, eicosenoic acid (C20:1) content and erucic acid (C22:1) content were identified in different linkage groups respectively and explain 10.22%-31.32% of phenotypic variation (Zhang et al., 2008). The additive main affects also were determined by QTL identification, it was indicated that interrelationship between fatty acid composition and oil content should be considered when breeding for increased oil content or improved oil composition in rapeseed (Jianyi et al., 2008).

Nowadays, identification of QTLs for tolerance to abiotic and biotic stress is becoming a hot research in rapeseed breeding. Such as freezing tolerance is a major component of winter survival and is the ability of plant to survive subfreezing temperatures. Four putative QTLs were detected and explained 24% of the low temperature total phenotypic variance by using single marker analysis (SMA), interval mapping (IM) and composite interval mapping (CIM) methods (Asghari et al., 2008). *Sclerotinia* stem rot is one of the most devastating diseases caused by fungus

Sclerotinia sclerotiorum. QTLs associated with *Sclerotinia* stem rot were identified in a total of eight genomic regions affecting resistance on N2, N12 linkage groups, and explained 6-22% of variance (Jianwei et al., 2006). QTL detected for resistance to *Sclerotinia sclerotiorum* in multiple environments was conducted by using a DH population. A genetic linkage map with 20 linkage groups covering 1746.5 cM, with an average space of 6.93 cM, was constructed using a total of 252 molecular markers. QTLs associated with resistance to *Sclerotinia sclerotiorum* were identified in N3, N4, N12 linkage groups (Xiangrui et al., 2010). Other researches focus on detection of QTLs associated with Turnip yellows virus (TuYV) which is responsible for a recognizable loss of yield in European winter oilseed rape cultivation, one major quantitative trait locus was found on linkage group MS17, explaining up to 50% of the phenotypic variation, by using the composite interval mapping approach (Dreyer et al., 2001). Study on pod shatter-resistant in rapeseed is becoming more interesting because of its suitable for mechanical harvesting. 70 associated SNPs were discovered and a major QTL for rapeseed pod shatter-resistance was found on chromosome A09 using deep sequencing a representative library and performing bulk segregant analysis (Zhiyong et al., 2012).

Other studies have focused on identification of QTL associated with the traits which are used to increase the value of the rapeseed crop. Phytosterols in rapeseed is used to enrich food products, and sinapate esters are limiting the utilization of rapeseed proteins in the feed industry, three QTL associated with phytosterols were detected, explaining 60% of the genetic variance, four QTL associated with sinapate ester content were detected, which explaining 53% of the genetic variance. Furthermore, there is a pleiotropic effect of the two erucic acid genes

on phytosterol and sinapate ester content; the effect of the alleles for low erucic acid content is to increase phytosterol and sinapate ester content (Amar et al., 2008). Enhanced tocopherol content has become an important objective in oilseed rape breeding because of its natural antioxidants and serving as important dietary nutrients. Using a mixed-model composite interval mapping approach between one and five QTL with additive and/or additive × environment interaction effects have mapped for α-, γ- and total tocopherol content, individual QTL and locus pairs contributing 7.5 - 29.2% of variance (Marwede et al., 2005), other QTL for a-tocopherol content have been mapped on chromosome A02 and can be used for marker assisted selection for α-tocopherol content in rapeseed (Endrigkeit et al., 2009).

2.9 Near-infrared spectroscopy

Near infrared spectroscopy (NIR) is a spectroscopic method that uses the near infrared region of the electromagnetic spectrum from about 700 nm to 2500 nm (Hoffmeyer et al., 1995). It is a fast and simple testing methods which is characterized by analysis for component without damaging the sample, those component should be contained C-H, N-H, O-H and S-H chemical bonding (Galtier et al., 2007, Rahim et al., 2013). The first molecular overtone produced at 1450 nm to 2050 nm, however the second and third molecular overtone produced at 1050 nm-1700nm and 700 nm-1050 nm in some substances, separately. The specific absorption spectrum fingerprint of the substance can be measured within the near infrared spectral bands by combination of those molecular overtone. The same near infrared spectrum (sample fingerprint) must be from the same material which is the principle of NIRS for quality measurement. Accurate analysis of certain parameter using NIRS depend on establish of tested

sample database or calibration curve which can be done by special software through scanning spectroscopy of samples using NIR combination with the accurate determination by traditional analysis method (such as: GC, HPLC, TKN, FIA).

Near infrared spectroscopy is widely applied in agriculture for determining the quality of forages, oilseeds, coffee, tea, fruits, vegetables, sugarcane, fats, meat, and other agricultural products (Downey et al., 1999, Kawano, 1994, Laporte et al., 1999, Marten et al., 1983, Nicolaï et al., 2007, Pandord et al., 1988, Prevolnik et al., 2004, Purcell et al., 2007). It is widely used to quantify the composition of agricultural products because it meets the criteria of being accurate, reliable, rapid, non-destructive, and inexpensive.

2.10 SPAD chlorophyll meter reading

The SPAD chlorophyll meter reading (SCMR) is a simple, portable diagnostic tool that measures the relative chlorophyll content of leaf (Kariya et al., 1982). Chlorophyll content of leaf determined by SCMR through measuring the absorbance of the leaf in two wavelength regions 400-500nm and 600-700nm regions with no absorbance in the near-infrared region (Ambrose, 2010). Using these two absorbances, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf.

The SPAD chlorophyll meter reading serves in a wide variety of plant applications and has established itself as a leading brand in chlorophyll measurement across the world (Azia et al., 2001, Balasubramanian et al., 2000, Ming et al., 2007, Pan et al., 2012, Uddling et al., 2007). It has been serving researchers and growers and enabling them to perform field tests quickly and easily. The SPAD chlorophyll meter

reading has been used for various kinds of plants and its applications are still growing since it need small measuring area with high accuracy. The measuring area is only 2×3 mm, allowing measurements of even small leaf.

2.11 Reference

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CHAPTER III

THE INHERITANCE ANALYSIS FOR LEAF CHLOROPHYLL AND ANTHOCYANIN CONTENTS IN RAPESEED

3.1 Abstract

Chlorophyll and anthocyanin are the most important pigments contributing to growth, yield and biotic stress adaptation in plants. Therefore, a detailed study on inheritance of anthocyanin and chlorophyll contents in rapeseed leaf were carried out in a 10-Zi006×10-4438 cross using generation mean analysis with RCB design in two years. Significant differences among generations for the traits indicated the presence of genetic variability between the parents. Anthocyanin content was significantly controlled by additive genes, while additive and dominance effects were both play an important role for genetic control of chlorophyll content, and these was confirmed by the narrow sense heritability of anthocyanin content (83.79% and 83.19%) which higher than chlorophyll content (50.59% and 35.96%) in both 2010-2011 and 2011-2012, respectively. The epistatic effect were important and higher than additive and dominance effects for both traits. The total fixable gene effects were higher than total non-fixable gene effects for the inheritance of anthocyanin content, but the vice versa result was found for chlorophyll content. On the other hand, both traits were affected significantly by environment. These reveal that selection for anthocyanin content in

early generations would be effective. Negative correlation between anthocyanin and chlorophyll contents (-0.12* to -0.26**) varies with generations indicating chlorophyll content in leaf were decreased due to the high concentration of anthocyanin.

3.2 Introduction

Anthocyanin, a type of flavonoid pigments, is commonly responsible for orange red to violet blue color in plant tissues (Tanaka et al., 2008). It was studied mostly on horticultural crops as a main target in breeding program due to some medicinal functions which have been suggested to reduce certain cancers, coronary heart diseases, oxidative stress and other age-related diseases (Shin et al., 2006, Williams et al., 2008). Therefore, the inheritance analysis for anthocyanin content in eggplant, ripe berries, strawberry had been carried out and defined the gene action for improvement of their nutritional quality (Liang et al., 2012, Sabolu et al., 2014, Singh et al., 2011). In addition, the anthocyanin plays an important role in breeding program of rapeseed (*Brassica napus* L.) since the purple red leaf could serve as a morphological marker (Wang et al., 2007). Development and application of hybrid is one of effective ways to increase the rapeseed yield. Several systems have been developed for utilization of heterosis in China, mainly are cytoplasmic male sterility (CMS) and genetic male sterility (GMS) (Fu et al., 1990, Liu et al., 2012). However, F_1 hybrid purity is one of the main constant in seed production since the pollens contamination by fertile plants in GMS system and self-pollination of sterile line in CMS system. Considerable works have been carried out such as three-line GMS and CMS + SI (self-incompatibility) systems suggested as effective methods to solve this

problem (Meng et al., 2009, Shen et al., 2008). On the other hand, breeding for restorer line or temporary maintainer line with purple red leaf for further development of rapeseed hybrid with morphological marker (purple red leaf) had been recommended to identify the false hybrid of F_1 (Wang et al., 2007, Wu et al., 2008). Through this method, the false hybrid line in seed production by pollens contamination could be eliminated earlier from the nursery bed. The rapeseed line "Zigan" with purple red leaf have been successfully developed by distant hybridization. This obvious color started at cotyledons stage, and change to green color differed from line to line. The purple red color disappeared in most rapeseed inbred line during the bolting stage and some lines could last to full-blossom stage (Wang et al., 2007). Moreover, the rapeseed cultivars with anthocyanin in leaf are classified as drought and frost resistant since this pigment have been proved to be the important function in photosynthetically active tissues of winter rapeseed involved in protection of mesophyll against cold-induced radiation stress (Solecka et al., 1999), and in ameliorating environmental stresses induced by visible and UV-B radiation, drought and cold temperature (Chalker - Scott, 1999). Therefore, the development of rapeseed cultivars with high anthocyanin is very useful for resistance to drought and cold temperature which are the main limiting factors in rapeseed production. However, the possible effects of anthocyanin were confounded by a decrease in photochemical efficiency with chlorophyll loss in senescing leaf of *Cornus sanguinea* and *Parthenocissus quinquefolia* (Manetas et al., 2011). Moreover, a significant decrease of photosynthetic pigments simultaneous with an increase of anthocyanin was measured in poinsettia bract development (Slatnar et al., 2013). Limited information is available on gene action for anthocyanin and chlorophyll contents in rapeseed leaf.

Since chlorophyll and anthocyanin are the most important plant pigments as they are necessary for photosynthesis drought and cold resistant mechanisms, so genetic control model for anthocyanin and chlorophyll contents would be useful for breeding program. The objective of this research were to, define the gene action for anthocyanin and chlorophyll contents, and investigate the correlation between them for further rapeseed hybrid breeding.

3.3 Materials and Methods

3.3.1 Plant materials and population development

Two inbred lines of rapeseed, 10-Zi006 and 10-4438, were used to develop the analysis generations. 10-Zi006 with purple red leaf was developed through interspecific hybridization using Youyan 2 (*B. napus*) as male and Hong youcai (*B. campestris*) as female; 10-4438, inbred line, with green leaf was developed by self-pollination of P6036-1. In the first season (2010/2011), two inbred lines were crossed to generate the F_1 population by hand emasculation and pollination techniques. In the second season (2011/2012), F_1 plants were selfed to develop F_2 generation and backcrossed to each parents to obtain BC_1 and BC_2 generations, respectively.

3.3.2 Experimental design

Six generations, P_1, P_2, F_1, F_2, BC_1 and BC_2, were evaluated in the experimental field at Guizhou Academy of Agriculture Sciences, Guizhou, China for two seasons (2012-2013 and 2013-2014) in a randomized complete block design with 3 replications. The seeds of 6 generations were sowed in nursery plots，and then transplanted at 35 days after planting into individual plot with rows of 240 cm in length. The spacing was 45 cm between rows and 30 cm between plants within row

with two plants per hill. Parental lines and F_1 generations were grown in six rows. For segregation generation in 2012-2013, F_2, BC_1 and BC_2 were represented by 27 rows, 21 rows and 12 rows, respectively. In the season of 2013-2014, each parents and F_1 generations were represented by six rows, F_2 represented by 21 rows, BC_1 and BC_2 represented by 12 rows, respectively.

3.3.3 Measurement of anthocyanin content

The anthocyanin content of individual plant in each generations were measured by photometric method (Mehrtens et al., 2005) at the seedling stage (with 6 to 7 leaf). The top expanded leaf were extracted at the same day for all plants in experiment. One milliliter of acidic methanol (1% HCl, w/v) was added to 300 mg of fresh leaf. The samples were incubated for 18 h at room temperature under moderate shaking, then sedimented by centrifugation (12710 ×g at room temperature for 1 minute) and 400 µl of the supernatant was added to 600 µl of acidic methanol. Absorption of the extracts at 530 and 657 nm wavelength were determined by a spectrophotometer (UV-7504, Shanghai, China). Quantification of anthocyanin was performed using the following equation:

$$Q_{\text{anthocyanin}} = (A_{530}-0.25\times A_{657})\times M^{-1}$$

Where $Q_{\text{anthocyanin}}$ is the amount of anthocyanin, A_{530} and A_{657} are the absorption at the indicated wavelengths and M is the weight of the rapeseed leaf used for extraction.

3.3.4 Measurement of chlorophyll content

Total chlorophyll content was determined by SPAD Chlorophyll Meter Reading (SCMR) in the same growth stage when the measurement of anthocyanin content. Each top expanded leaf were measured five times in different parts to

monitor the chlorophyll status. SCMR was recorded using a chlorophyll content meter (Minolta SPAD-502 plus, Osaka, Japan)

3.3.5 Genetic analysis

A, B and C scaling test according to Mather (1949) and Hayman and Mather (1955) were calculated for the absence or presence of non-allelic interactions. Joint scaling test proposed by Cavalli (1952) was used to estimate the parameters of m, d and h, and test the goodness of fit of the additive-dominance model by χ^2 testing. This simple genetic model was adequate when epistasis was absent, whereas the six parameters model were applied for estimation of various genetic components since the presence of non-allelic interaction. Estimated of six parameters were performed according to Hayman (1958). Broad sense and narrow sense heritability were estimated using the method outlined by Warner (1952). R software was used for data analysis.

3.4 Result and Discussion

3.4.1 Variations of generation means

The test of homogeneity ($F_{\text{anthocyanin content}} = 1.37^{ns}$, $F_{\text{chlorophyll content}} = 1.19^{ns}$) showed that the data of both study traits could be combined analysis over two years. The analysis of variance of generation means for anthocyanin and chlorophyll contents from two seasons were combined and shown in Table 3.1. There were no significant difference between replications on both anthocyanin and chlorophyll contents. However, the anthocyanin content were significant and chlorophyll content were highly significant between years. This indicates that environment affected both studied traits significantly and maybe due to the environmental factors such as UV-A

and UV-B regulated the synthesis of anthocyanin in leaf (Tossi et al., 2011, Wang et al., 2012). On the other hand, there were highly significant difference between generations on studied traits, especially between parental lines (Fig.3.1, 3.2), the high significant difference on anthocyanin content among genotypes was also founded in strawberry and the synthesis were significantly affected by environment (Singh et al., 2011). Moreover, the environment (year) × generations interaction was significant difference for anthocyanin content but not significant for chlorophyll content. The anthocyanin content in BC_1 generation was skewed towards the P_1. However, the generation mean in F_1, F_2 and BC_2 were skewed towards to P_2 (Fig. 3.1). The means of chlorophyll content in P_1 was significantly higher than other generations, and the means of F_1, F_2, BC_1 towards to the mid-parents. However, the mean of chlorophyll content in BC_2 was skewed towards the high value parent P_1 (Fig. 3.2). The results were quite similar in both seasons.

Table 3.1 Analysis of variance for anthocyanin and chlorophyll contents of P_1, P_2 and F_1, F_2, BC_1, BC_2 generations evaluated in 2012-2013 and 2013-2014 at Guiyang, Southwest of China

Source of variation	df	Mean square	
		AC	CC
Replication	2	0.002	0.711
Years	1	0.232*	203.633**
Generations	5	3.634**	60.871**
Years × Generations	5	0.024*	0.322
Error	22	0.006	0.919
Total	35		

AC=Anthocyanin content; CC=Chlorophyll content; *P <0.05; **P <0.01

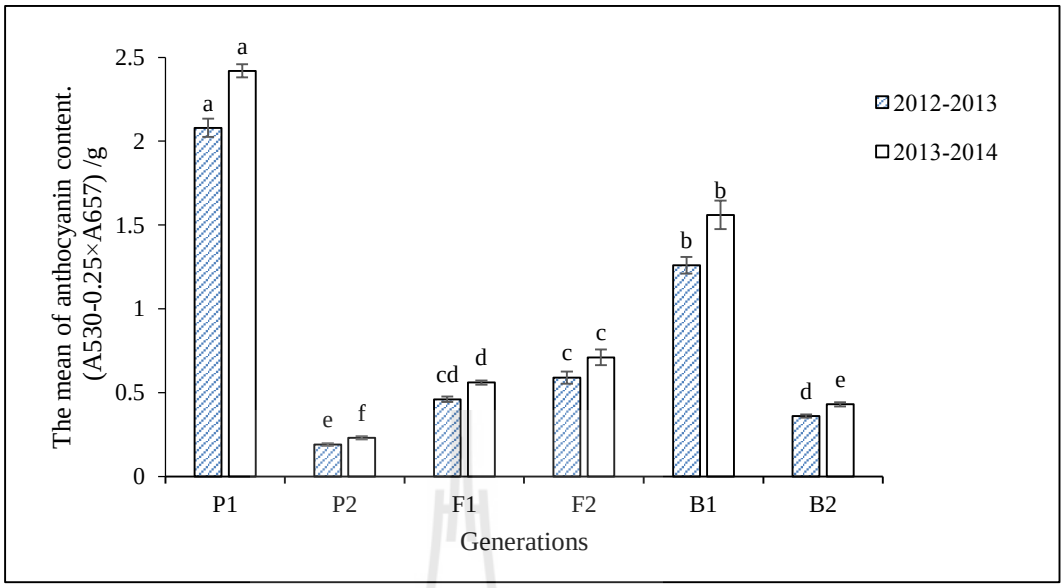

Figure 3.1 Generation means and standard errors of anthocyanin content in 2012-2013 and 2013-2014

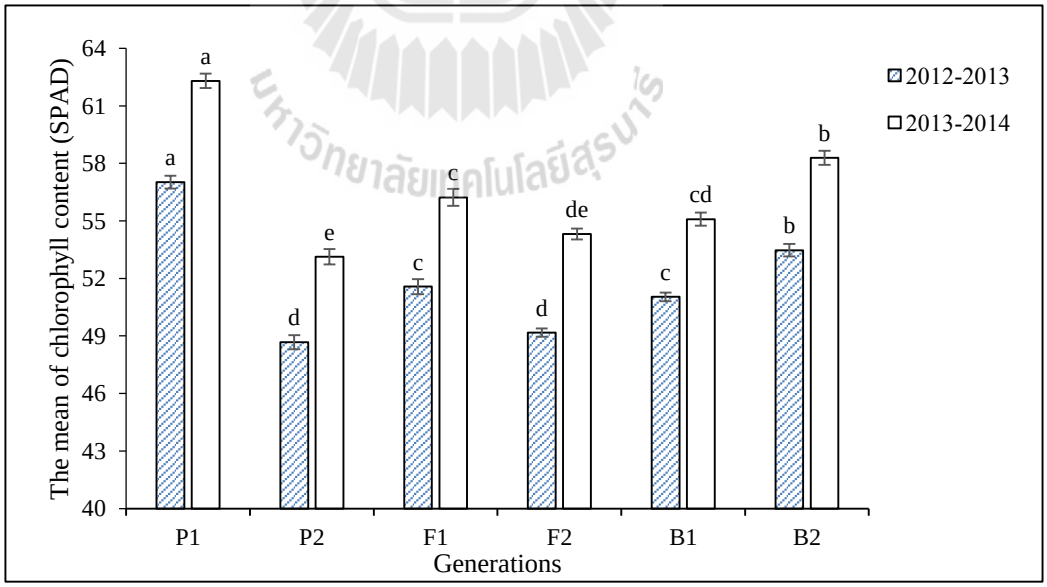

Figure 3.2 Generation means and standard errors of chlorophyll content in 2012-2013 and 2013-2014.

3.4.2 Additive-dominance model

The result for the additive-dominance model testing of anthocyanin and chlorophyll contents were shown in Table 3.2. Highly significant difference of *t*-test has been detected between parents for both traits indicating that the data was suitable for genetic analysis. The scaling test and joint scaling test for additive-dominance genetic model were also presented. The significant and highly significant different of B scaling for anthocyanin content were found in 2013 and 2014, respectively. C scaling was highly significant and A scaling were not significant difference in both two consecutive years for anthocyanin content. However, A, B and C scaling were found to be highly significant for chlorophyll content in both years. Single scaling test indicated additive-dominance model were inadequate for anthocyanin and chlorophyll contents and reveal of non-allelic interaction. The joint scaling test confirmed absolutely the results in single scaling test since the χ^2 vales were highly significant for all models.

Table 3.2 Estimates of t-test, scaling tests and joint scaling test ($\chi 2$) for AC and CC

Scaling test	**AC**		**CC**	
	2012-2013	**2013-2014**	**2012-2013**	**2013-2014**
t-value	34.04**	32.77**	16.84**	16.80**
A	-0.02±0.115	0.14±0.176	– 6.51**±0.679	-8.34**±0.890
B	0.07**±0.027	0.07*±0.029	6.70**±0.849	7.23**±0.938
C	-0.83**±0.157	-0.93**±0.195	–12.15**±1.275	-10.63**±1.530
$\chi 2$	39.25**	31.54**	305.62**	243.30**

AC=Anthocyanin content; CC=Chlorophyll content; t-value=t test for parents; *P <0.05; **P <0.01

3.4.3 Estimates of gene action

Types of gene action estimated by generation mean as genetic effects in six parameter model are presented in Table 3.3. The estimated values for mean effects (m) were highly significant for both studied traits indicating that anthocyanin and chlorophyll contents in rapeseed were quantitatively inherited. The additive effect [d] was highly significant and positive for anthocyanin content. Contrarily, negative additive effect for chlorophyll content was also significant and the same results were obtained in both two seasons. The dominance effect [h] was not significant for anthocyanin content but positive effect was highly significant for chlorophyll content in both 2013 and 2014. High significant epistatic additive × additive type of gene effects [i] were detected for anthocyanin and chlorophyll contents in both years. Additive × dominance [j] were found to be high negative significant for chlorophyll content but was not significant for anthocyanin content, and the result were similar in both two years. Dominance × dominance effect [l] were negative significant for anthocyanin content and highly negative significant for chlorophyll content both in 2013 and 2014. The previous research results differ from gene action for inheritance of anthocyanin content in rapeseed leaf. Some researchers reported that the additive gene effect was significant for genetic control of anthocyanin content in grape, eggplant and a simple additive-dominance genetic model was adequate both for anthocyanin and chlorophyll contents in pepper fruit (Liang et al., 2012, Sabolu et al., 2014). In this present research, the additive gene effect also was the mainly genetic effect that controlled the inheritance of the anthocyanin content in rapeseed. However the simple additive-dominance genetic model was inadequate for the inheritance of anthocyanin content. On the other hand, the additive gene action as main genetic

effect for inheritance of anthocyanin content were supported by higher heritability (Liang et al., 2012, Sabolu et al., 2014, Singh et al., 2011). In contrast with anthocyanin content, the dominance effect was the main genetic effect of the inheritance of chlorophyll content (Farshadfar et al., 2011). Our results also revealed high magnitude of dominant effect, additive x dominance effect and dominance × dominance effect in inheritance of chlorophyll content.

Table 3.3 Estimates of the components of the generation means for a six-parameter model

Parameters	**AC**		**CC**	
	six-parameter model		**six-parameter model**	
	2012-2013	**2013-2014**	**2012-2013**	**2013-2014**
m	0.590**±0.036	0.710**±0.047	49.170**±0.218	54.29**±0.7812
[d]	0.900**±0.051	1.130**±0.086	–2.430**±0.396	–3.20**±0.6491
[h]	0.205±0.179	0.375±0.257	11.065**±1.266	8.11**±3.4279
[i]	0.880**±0.176	1.140**±0.255	12.340**±1.178	9.60** ±3.3838
[j]	–0.045±0.058	0.035±0.089	–6.605**±0.467	–7.78**±0.7630
[l]	-0.903*±0.258	-1.35*±0.396	–12.530**±2.033	–8.49**±4.2080

AC=Anthocyanin content; CC=Chlorophyll content; *P <0.05, **P <0.01; m= mean of the generation; [d]= additive effect; [h]= dominance effect; [i]= additive × additive effect; [j]= additive × dominance effect; [l]= dominance × dominance effect.

The magnitude of gene effects (%) for both anthocyanin and chlorophyll contents were slightly differ from seasons (Table 3.4). The greatest magnitude of gene

effects for anthocyanin content was dominance × dominance [l] followed by additive [d] and additive × additive [i] in 2013. However, dominance × dominance [l] was the greatest followed by additive × additive [i] and additive [d] in 2014. The greatest magnitude of gene action for chlorophyll content was dominance × dominance [l] followed by additive × additive [i] and dominance [h] in 2013, and was additive ×additive [i] followed by dominance × dominance [l] and dominance [h] in 2014.

Table 3.4 Relative magnitude (%) of gene effect s for anthocyanin and chlorophyll contents

Parameters	**AC**		**CC**	
	2012-2013	**2013-2014**	**2012-2013**	**2013-2014**
m	100.00	100.00	100.00	100.00
[d]	152.54**	159.15**	-4.94**	-5.89**
[h]	34.75	52.82	22.5**	14.94**
[i]	149.15**	160.56**	25.1**	17.68**
[j]	-7.63	4.93	-13.43**	-14.33**
[l]	-153.05*	-190.14**	-25.48**	-15.64**

AC=Anthocyanin content; CC=Chlorophyll content; *P <0.05; **P <0.01; m= mean of the generation; [d]= additive effect; [h]= dominance effect; [i]= additive × additive effect; [j]= additive × dominance effect; [l]= dominance × dominance effect.

The generation means analysis revealed that epistatic effects were higher than the main effects for studied traits. This result demonstrated that non-allelic

interactions played an importance role in the genetic control of both traits especially the chlorophyll content in rapeseed leaf. On the other hand, the total fixable gene effects was higher than total non-fixable gene effects in the genetic control of anthocyanin content, but the contrast result was found for chlorophyll content both in two seasons (Table 3.5). These information provided a breeding strategy on improvement of anthocyanin and chlorophyll contents in rapeseed that breeding for anthocyanin content could be selected in early generations and breeding for chlorophyll content must use complicated procedure for their further exploitation.

Table 3.5 The decomposition of gene effects for anthocyanin and chlorophyll contents in six rapeseed generations grown in two years

Characters		Main effects		Epistatic	Total gene effects	
		[d]	[h]	effects	Fixable	Non-fixable
AC	2012-2013	0.900	0.205	1.828	1.78	1.153
	2013-2014	1.130	0.375	2.525	2.27	1.76
CC	2012-2013	2.430	11.065	31.475	14.77	30.2
	2013-2014	3.200	8.110	25.87	12.8	24.38

AC=Anthocyanin content; CC=Chlorophyll content; Epistatic effects= [i] + [j] + [l]; Fixable components= [d] + [i]; Non-fixable components= [h] + [j] + [l] (ignoring signs).

The types of gene action estimated considering genotype × environment interaction as twelve parameters model were presented in Table 3.6. The additive effect, additive × additive effect and dominance × dominance effect were highly

significant, and environmental effect and environmental × additive effect were significant in genetic model of anthocyanin. However, the additive effect, dominance effect, additive × additive effect, additive × dominance effect, dominance additive × dominance effect and environmental effect were highly significant in the genetic model of chlorophyll in rapeseed leaf. On the other hand, the test of fitness of model indicated that the m+ [d] + [h] + [i] + [l] + el + gdl + gil and m+ [d] + [h] + [i] + [j] + [l] + el were the perfect model for anthocyanin and chlorophyll inheritance in rapeseed, respectively, since the Chi-square test of both were not significant.

Table 3.6 The genotype × environment interaction for anthocyanin content and chlorophyll content in six generations of the cross 10-4438 × 10- Zi006 grown in two years

Parameters	AC		CC	
	Full model	**Fitness of model**	**Full model**	**Fitness of model**
m	0.650**±0.030	0.650**±0.029	51.740**±0.178	51.691**±0.174
[d]	1.015**±0.050	1.017**±0.016	-2.815**±0.318	-2.672**±0.307
[h]	0.290±0.157	0.291*±0.120	9.545**±1.016	10.029**±0.985
[i]	1.010**±0.155	1.009**±0.122	10.930**±0.954	11.396**±0.922
[j]	-0.005±0.053	-	-7.195**±0.367	-7.03**±0.358
[l]	-1.140**±0.236	-1.144**±0.135	-10.470**±1.614	-11.22**±1.568
e1	0.060*±0.030	0.058**±0.007	2.570**±0.178	2.373**±0.094
gd1	0.115*±0.050	0.075**±0.015	-0.385±0.318	-
gh1	0.085±0.157	-	-1.520±1.016	-
gi1	0.130±0.155	0.038*±0.016	-1.410±0.954	-
gj1	0.040±0.053	-	-0.590±0.367	-
gl1	--0.210±0.236	-	2.060±1.614	-
χ2(df)	-	2.17 (4)^{ns}	-	5.66 (5)^{ns}

AC= Anthocyanin content; CC= Chlorophyll content; ^{ns} No significant at P=0.05; *P <0.05; **P <0.01; m= mean of the generation; [d]= additive effect; [h]= dominance effect; [i]= additive × additive effect; [j]= additive × dominance effect; [l]= dominance × dominance effect; el= environmental effect; gdl= environmental × additive effect; ghl= environmental × dominance effect; gil= environmental × additive × additive effect; gjl= environmental × additive × dominance effect; gll=environmental × dominance × dominance effect.

3.4.4 Heritability

The estimates of heritability for anthocyanin and chlorophyll contents were presented in Table 3.7. High broad sense heritability and moderate narrow sense heritability were obtained for anthocyanin content indicating that indicating this character was controlled mainly by genetic effect, and both additive and non-additive gene effects were important. However, moderate broad sense heritability and lower narrow sense heritability were detected for chlorophyll content indicating that this trait was affected both by genetic effect and non-genetic effect and the additive effect and non-additive effect as well as environmental effect were important.

3.4.5 Correlation between anthocyanin and chlorophyll contents

Relationships between anthocyanin and chlorophyll contents were recorded in P_1, P_2, F_1, F_2, BC_1 and BC_2 generations, each generation with 80, 78, 75, 418, 318, 156 plants in 2012-2013 and 75, 66, 69, 301, 160 and 156 plants in 2013-2014, respectively. The negative correlation between anthocyanin and chlorophyll contents have been obtained in P_1, F_2 and BC_1 generations in both two seasons. Especially, highly significant negative correlation obtained in BC_1 generation in both seasons and in F_2 generation in 2012/. However, no significant positive correlation were detected in P_2, F_1 and BC_2 generations. In accordance with our studies, the negative correlation and positive correlation between anthocyanin and chlorophyll contents was observed in the generation with purple-red leaf (P_1) and with green leaf (P_2), respectively. The same relationship were detected between black and violet pepper groups indicating chlorophyll content in plant were decreasing due to the high concentration of anthocyanin (Stommel et al., 2014). In addition, the chlorophyll content in all the segregating generations were detected to be lower

negative correlation with anthocyanin content in rapeseed leaf. In contrast with segregating generations, positive correlation was observed between anthocyanin and chlorophyll content in F_1 generation. This result was useful for breeding of rapeseed hybrid with purple-red leaf and resistance to drought and cold temperature while with higher photosynthetic capacity.

Table 3.7 Estimates of heritability for anthocyanin and chlorophyll contents

Parameters	**AC**		**CC**	
	2012-2013	**2013-2014**	**2012-2013**	**2013-2014**
h_B^2	83.79	83.19	52.75	54.37
h_N^2	50.59	35.56	34.92	36.46

h_B^2= Broad sense heritability; h_N^2= Narrow sense heritability.

3.5 Conclusion

The inheritance of anthocyanin and chlorophyll contents in rapeseed leaf did not follow the simple additive-dominance genetic model. The main gene effect, non-allelic interactions and environmental effect controlled the traits significantly. The magnitude of additive effect for anthocyanin content and magnitude of dominance effect for chlorophyll content provided a breeding strategy that selecting for anthocyanin content in early generations were effective, however, backcross, SSD/pedigree selection was a tool for improvement of chlorophyll content.

Table 3.8 Pearson correlation coefficients for anthocyanin and chlorophyll contents in each P_1, P_2, F_1, F_2, BC_1 and BC_2 generation.

Generations	2012-2013	2013-2014
P_1	-0.15	-0.19
P_2	0.16	0.15
F_1	0.07	0.08
F_2	-0.18^{**}	-0.12^{*}
BC_1	-0.26^{**}	-0.26^{**}
BC_2	-0.08	-0.01

^{ns} No significant at P=0.05; *P <0.05; **P <0.01

3.6 Reference

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CHAPTER IV

THE INHERITANCE ANALYSIS FOR SEED OIL CONTENT IN RAPESEED

4.1 Abstract

Improvement of seed oil content is an important objective in rapeseed breeding due to its economic value. To get better knowledge on gene action for oil content, a genetic study with six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) derived from a 10-4438 × 10- Zi006 cross were conducted in a RCB design with 3 replications. Two analysis methods, generation mean and major gene plus polygene analysis, were employed to investigate the gene action of oil control in rapeseed. The results shown that oil content was controlled significantly by additive effects, followed by dominance × dominance epistatic effects. Furthermore, E-1 model, a mixture of two major additive-dominant-epistasis genes plus additive-dominant polygenes, was determined to be the best-fitting model to explain the inheritance of oil content. The dominance × dominance epistatic effects between two major genes was the main gene effect while both additive effect and dominance effect of polygenes have a magnitude of effects. The major genes can explain 57.78%, 39.27% and 27.23% of the phenotypic variance and 99.70%, 82.20% and 41.10% of genetic variance in BC_1, BC_2 and F_2 generation, respectively.

4.2 Introduction

Rapeseed (*Brassica napus* L.) is an important source of vegetable oil for human nutrition. Improving of oil content was emphasized by breeders as an important objective in rapeseed breeding program. Therefore, a better understanding of gene action for inheritance of oil content would be important in helping the breeder to control the genetic advance. Oil content in rapeseed is a complex quantitative trait controlled by multiple genes and influenced by environmental factors (Si et al., 2003, Zhao et al., 2005). This trait varies quantitatively among germplasm of rapeseed due to the complex regulation of multiple genes on metabolism of seed oil (Barker et al., 2007). The genetic control of oil content in rapeseed was reported differently under specific materials. Additive effect has been detected playing a major role on controlling the oil content in rapeseed (Delourme et al., 2006, Shen et al., 2005, Variath et al., 2009, Zhang et al., 2006). In addition, additive-dominant-epistasis genetic model was defined controlling the oil content with significance of dominant effect, additive effect and epistatic effect (Wang et al., 2010). Moreover, genetic analysis using major gene plus polygene method revealed oil content in rapeseed controlled by genetic model of one additive major gene plus additive and dominance polygenes (Zhang et al., 2006). On the other hand, QTL identification for oil content indicated additive effects and epistatic as well as environmental interactions played an important role in inheritance of oil content in rapeseed (Zhao et al., 2005)

10-Zi006 is a rapeseed inbred line with purple-red leaf which color started from cotyledons stage. This specific trait is very useful in identification of hybrids of rapeseed. Moreover, it have an ability of resistance to cold temperature due to the existing of anthocyanin (Chalker-Scott, 1999, Solecka et al., 1999). However, seed oil

content in 10-Zi006 was lower about 33.5% comparing to other varieties. To understand the gene action for inheritance of oil content, the inbred line of 10-Zi006 and 10-4438 which with green leaf and high oil content in seeds, were selected as parents to develop six generations. The generation mean and major gene plus polygene analysis were employed, and a clear genetic model is proposed for the genetic improvement of oil content in rapeseed with purple-red leaf.

4.3 Materials and Methods

4.3.1 Plant materials and population development

Two inbred lines of rapeseed, 10-Zi006 and 10-4438, were used to develop the analysis generations. 10-Zi006 with purple-red leaf was developed through interspecific hybridization using Youyan 2 (*B. napus*) as male and Hong youcai (*B. campestris*) as female. 10-4438 with green leaf was developed by self-pollination of P6036-1. In the first season (2010/2011), two inbred lines were crossed to generate the F_1 population by hand emasculation and pollination techniques. In the second season (2011/2012), F_1 plants were selfed to develop F_2 generation and backcrossed to two parents to obtain BC_1 and BC_2 generations, respectively.

4.3.2 Experimental design

Six generations, P_1，P_2，F_1，F_2，BC_1 and BC_2, were evaluated at Guizhou Academy of Agriculture Sciences (GZAAS) experimental field on 2012-2013 using a randomized complete blocks design with 3 replications. The seeds of six generations were sowed in nursery plots，and transplanted 35 days after planting into individual plot with rows of 240 cm in length, with 45-cm inter-row spacing and 30-cm intra-row spacing, and with two plants per hill．Parental lines and F_1 generations were grown in

six rows. For segregation generation, F_2, BC_1 and BC_2 were represented by 27 rows, 21 rows and 12 rows, respectively.

4.3.3 Measurement of oil content

Percentage oil content (OC%, w/w) in rapeseed seeds of individual plant in each generation was analyzed by near-infrared reflectance spectroscopy (NIRs, Foss NIRSystems 3700). However, the oil content of 103 individuals in BC_1 population was measured by both NIRs and Soxhlet extraction methods. The oil content analyzed by NIRs were then calibrated through an equation which was established using stepwise multiple linear regression (SMLR) method.

Soxhlet extraction was carried out using petroleum ether as the solvent. Rapeseed powder (2.0 g) with mesh size of 60 was weighed (A). The sample was then roasted for 3 h at 105 ˚C in hot air oven and cooled down for 30 min. The resulting sample was then weighed (B). The rapeseed oil was then extracted by a Soxhlet extractor. The Soxhlet extraction process took about 6 h, with an extraction temperature of 100℃. After reaction, the filter bag was baked for 30 min and weighed (C). Oil extraction rate was calculated, and oil percentage (w/w) was obtained with respect to the total oil present in rapeseed using follow equation (Martínez et al., 2008).

$$Y=(B - C) / A \times 100$$

4.3.4 Genetic analysis

A, B and C scaling test (Hayman et al., 1955, Mather, 1949) was used to provide information regarding absence or presence of non-allelic interactions. Joint scaling test (Cavalli, 1952, Mather et al., 1982) was used to estimate the parameters of

m, d and h and test the goodness-of-fit of the additive-dominance model by testing (χ^2), and this simple genetic model was adequate when epistasis was absent, whereas the six parameters model were applied for estimation of various genetic components since the presence of non-allelic interaction. Broad sense and narrow sense heritability were estimated according to the commonly formula (Warner, 1952).

4.3.5 Major gene plus polygene analysis

Mixed major gene plus polygene analysis (Akaike, 1977, Cao, 2013, Gai et al., 2003) was employed to evaluate the maximum likelihood of various possible genetic models. Maximum likelihood evaluation and IECM (iterated expectation and conditional maximization) were conducted to evaluate the parameters of the generations. AIC (akaike information criterion) provided a means for better models selection. Then, a series of goodness-of-fit tests including uniform test (U_1^2, U_2^2 and U_3^2), Smimov test ($_nW^2$) and Kolmogorov test (D_n) were conducted to select the optimal genetic model. Finally, least squares method was adopted to evaluate the contribution rates, variance and related genetic parameters of the genes in the optimum model. The analysis software (SEA) was provided by Nanjin agricultural university.

4.4 Result and Discussion

4.4.1 Calibration analysis of oil content

Foss NIRSystems 3700 near-infrared reflectance spectroscopy (NIRs) was used for measurement of the percentage oil content (OC%, w/w) of rapeseed seeds in Guizhou Oil Crops Institute, GZAAS every year. In this study, the oil content of dry rapeseed from 103 individual plants was analyzed using Soxhlet extraction and NIR

methods simultaneously. A calibration regression equation for oil content analyzed by NIRs was established as y=4.1945+0.8696x with a standard error of calibration as low as 0.053 and a coefficient of determination as high as 0.9554 (Figure. 4.1).

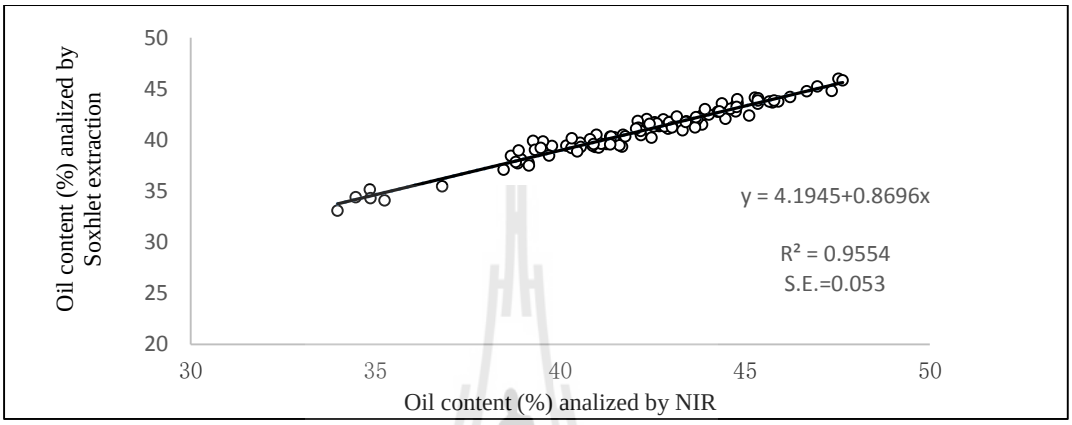

Figure 4.1 The correlation of oil content between NIR and Soxhlex extraction methods

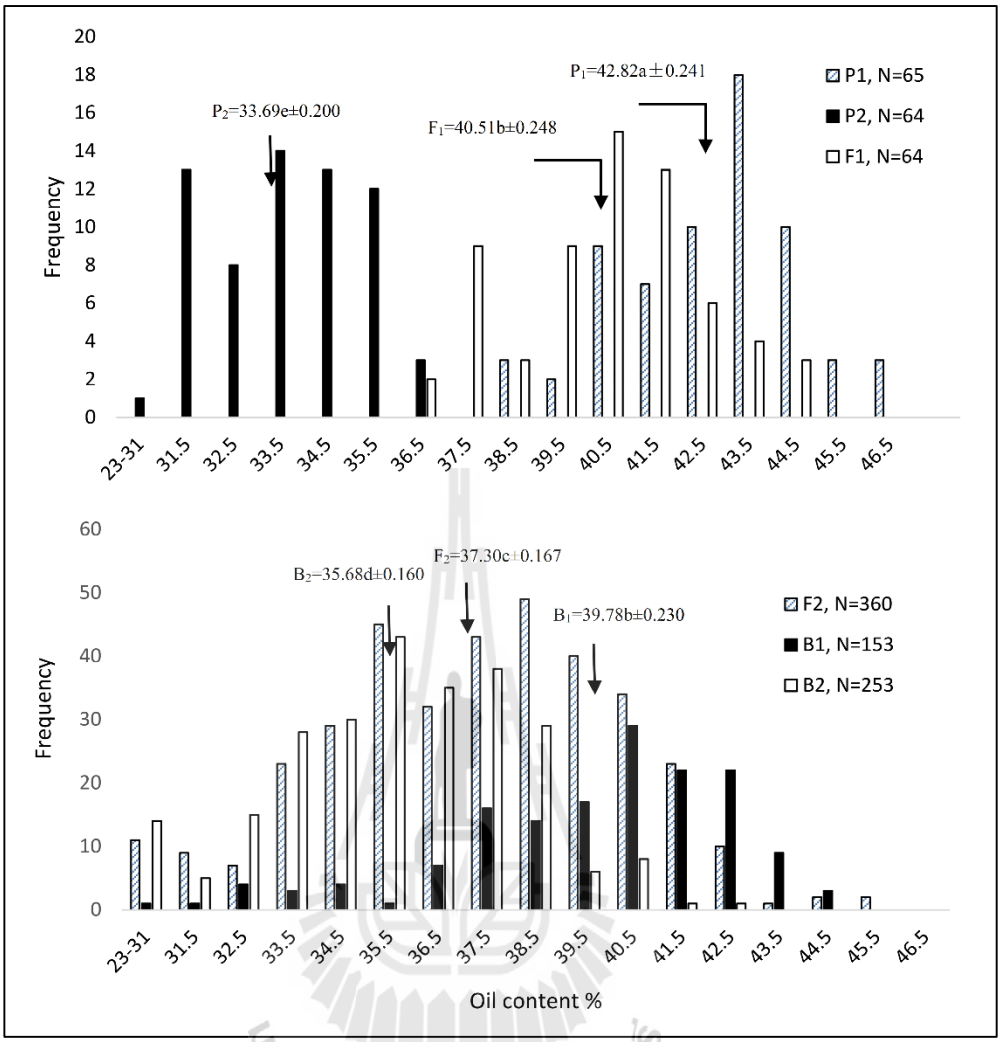

Figure 4.2 Frequency distribution of oil content for six generations of 10-4438 × 10-Zi006; Arrows indicate the position of mean followed by the mean and standard error of mean for the generation; a, b, c, d and e indicate the significance sign of "Duncan" multiple comparisons for the mean of generations

4.4.2 Variations of generation means

Frequency distributions of oil content was shown in Figure 4.2. The significant difference on oil content was detected between each generation except

between F_1 and BC_1. Two parental inbred lines differed significantly with average of 42.82% for 10-4438 (P_1) and 33.69% for 10-Zi006 (P_2). Oil content on F_1 and BC_1 were skewed towards the P_1 generation while the mean of F_2 was similar to mid-parents (38.26%), and BC_2 was skewed towards to P_2. On the other hand, multiple peaks were detected on the continuous distribution of BC_1, BC_2 and F_2 indicating the inheritance of oil content in rapeseed controlled by major genes plus polygenes.

4.4.3 Generation mean analysis

High significance of A, B and C scaling test indicated that simple additive-dominance genetic model was inadequate and non-allelic interaction governed the inheritance of oil content in rapeseed. Joint scaling test for additive-dominance model confirmed absolutely the results in single scaling test since the χ^2 vales (105.31) was highly significant and the parameters (m, d and h) were also highly significant (Table 4.1). Six parameters revealed additive effect contributed high significantly to the inheritance of oil content, followed by dominance × dominance epistatic effect. The further test for best-fitting genetic model shown that four parameters model (m + [d] + [i] + [l]) was adequacy because the mean effect (m), additive effect, additive × additive effect and dominance × dominance epistatic effect were significance, and the Chi-square value was not significance (Table 4.1). The others genetic models were inadequate because Chi-square vale was significant in three parameters model and dominance effect and additive × dominance epistatic effect were not significant in five parameters model. The broad sense heritability was higher than narrow heritability indicating inheritance of oil content was controlled mostly by additive gene effect.

Table 4.1 Scaling test of genetic models for oil content in rapeseed cross 10-4438 × 10- Zi006

Individual scaling test			**Heritability**	
A	**B**	**C**	**h_B^2**	**h_N^2**
-3.77**±0.575	**-2.84**±0.452**	**-8.33**±0.889**	**0.66**	**0.59**
Joint scaling test				
Parameters	Three parameters	Four-parameters	Five-parameters	Six-parameters
m	37.58**±0.141	36.22**±0.199	36.18**±0.202	36.54**±0.886
[d]	4.44**±0.136	4.46**±0.136	4.56**±0.156	4.57**±0.157
[h]	1.23**±0.273			-0.92^{ns}±2.212
[i]		2.01**±0.266	2.06**±0.269	1.72^{ns}±0.872
[j]			-0.43^{ns}±0.311	-0.465^{ns}±0.321
[l]		4.29**±0.386	4.32**±0.387	4.89*±1.430
χ2(df)	105.31**(3)	2.102^{ns}(2)	0.171^{ns}(1)	

^{ns} No significance; ** significance at P≤0.01, * significance at P≤0.05.

4.4.4 Major gene plus polygene analysis

The estimates of maximum log likelihood value (MLV) and AIC value in each genetic model were shown in Table 4.2. The model E-1 had the smallest AIC value, followed by the model E-0. Based on Akaike's hypothesis on maximizing expected entropy (Akaike, 1977), these models with the two lowest AIC values were chosen as candidates genetic model. Subsequently, the results of a series of tests including equal distribution test (U_1^2, U_2^2, U_3^2), Smirnov test ($_nW^2$) and Kolmogorov test (D_n) indicated model E-1 was the best-fitting genetic model to explain the inheritance of oil content (Table 4.3). According to the mixed inheritance models (Gai

et al., 2003), a mixture of two additive-dominant-epistasis major genes plus additive-dominant polygenes controlled the inheritance of oil content in 10-4438 × 10-Zi006 cross.

Table 4.2 The Values of maximum likelihood and AIC analyzed by IECM methods using six generations of cross 10-4438 × 10- Zi006.

Model	Code	MLV	AIC
A-1	1MG-AD	-2384.74	4777.48
A-2	1MG-A	-2388.54	4783.08
A-3	1MG-EAD	-2470.42	4946.85
A-4	1MG-AEND	-2523.55	5053.09
B-1	2MG-ADI	-2295.50	4611.00
B-2	2MG-AD	-2366.58	4745.16
B-3	2MG-A	-2426.09	4860.17
B-4	2MG-EA	-2362.07	4730.14
B-5	2MG-AED	-2410.04	4828.09
B-6	2MG-EEAD	-2410.04	4826.09
C-0	PG-ADI	-2299.35	4618.71
C-1	PG-AD	-2346.48	4706.96
D-0	MX1-AD-ADI	-2289.22	4602.44
D-1	MX1-AD-AD	-2351.10	4720.20
D-2	MX1-A-AD	-2336.23	4688.46
D-3	MX1-EAD-AD	-2343.81	4703.62

Table 4.2 (Continued) The Values of maximum likelihood and AIC analyzed by IECM methods using six generations of cross 10-4438 × 10- Zi006.

Model	Code	MLV	AIC
D-4	MX1-AEND-AD	-2344.54	4705.09
E-0	MX2-ADI-ADI	-2276.55	4589.09
E-1	MX2-ADI-AD	-2276.8	4583.61
E-2	MX2-AD-AD	-2345.29	4712.59
E-3	MX2-A-AD	-2325.12	4668.23
E-4	MX2-EA-AD	-2345.20	4706.40
E-5	MX2-AED-AD	-2342.55	4703.09
E-6	MX2-EEAD-AD	-2345.28	4706.55

MLV: maximum likelihood-value; AIC: Akaike's information criterion; MG: major gene model; PG: polygene model; MX1: mixed model of one pair of major gene plus polygene; MX2: mixed model of two pairs of major gene plus polygene; A: additive effect; D: dominance effect; I: interaction (epistasis); N: negative; E: equal.

Table 4.3 Test of goodness of fit for B-1, D-0, E-0 and E-1 models.

Model	Generations	U_1^2	U_2^2	U_3^2	${}_nW^2$	D_n
E-1	P_1	0.920(0.338)	1.046(0.306)	0.142(0.706)	0.257(0.186)	0.019(1.000)
	F_1	1.426(0.233)	1.555(0.212)	0.133(0.716)	0.264(0.178)	0.009(1.000)
	P_2	0.263(0.608)	0.679(0.410)	1.712(0.191)	0.138(0.431)	0.044(0.999)
	BC_1	0.090(0.764)	0.351(0.554)	1.450(0.229)	0.090(0.651)	0.017(1.000)
	BC_2	0.000(0.998)	0.033(0.855)	0.548(0.459)	0.058(0.829)	0.003(1.000)
	F_2	0.022(0.881)	0.114(0.736)	0.590(0.442)	0.050(0.876)	0.002(1.000)
E-0	P_1	0.126(0.722)	0.15(0.699)	0.029(0.865)	0.157(0.371)	0.022(1.000)
	F_1	0.049(0.826)	0.053(0.818)	0.005(0.947)	0.094(0.627)	0.013(1.000)
	P_2	0.005(0.945)	0.150(0.699)	1.637(0.201)	0.113(0.532)	0.050(0.994)
	BC_1	0.348(0.555)	0.278(0.598)	0.031(0.861)	0.131(0.456)	0.015(1.000)
	BC_2	0.012(0.913)	0.138(0.711)	1.130(0.288)	0.080(0.705)	0.002(1.000)
	F_2	0.127(0.721)	0.017(0.898)	0.751(0.386)	0.076(0.725)	0.002(1.000)

U_1^2, U_2^2, U_3^2: refer to uniformity tests, ${}_nW^2$: the Smirnov test statistic, D_n: the Kolmogorov test statistic. The numbers in parentheses refer to probabilities

The estimates of parameters for E-1 genetic model were shown in Table 4.6. The additive effect of the first-major-gene was equal with the second-major-gene (d_a = d_b = -0.16). The dominant effect had magnitude both for two major genes due to dominance ratio of the first- and second-major-gene were 15.93 and 11.25, respectively. On the other hand, the interaction effects between two major genes were positive to oil content except the effect of dominant × additive (j_{ba}). The dominance × dominance effect between two genes was contributed significantly to the inheritance of oil content. In addition, a magnitude of the additive effect and dominance effect of polygenes were contributed to the inheritance of oil content with the additive effect higher than dominance effect (Table 4.4). The variance of major genes accounted for

99.70%, 82.20% and 41.10% of genetic variance in BC_1, BC_2 and F_2 generation, respectively. In contrast, the polygenes explained 0.3%, 17.8% and 58.9% of genetic variance in BC_1, BC_2 and F_2 generation, respectively. The heritability of major gene in BC_1, BC_2 and F_2 generation was 57.80%, 39.30% and 27.20%, and the heritability of polygenes were detected as 0.20% and 8.50%, 39.00% in those generation, respectively. The results indicated oil content affected by environmental effects, and a magnitude of polygenes effects played an important role in F_2 generation.

Table 4.4 Estimates of parameters for the genetic models (E-1).

First order parameter				Second order parameter	Estimate		
Parameter	**Estimate**	**Parameter**	**Estimate**		**BC_1**	**BC_2**	**F_2**
m	37.00	i	1.13	σ^2_P	8.093	6.511	10.071
d_a	-0.16	$j_{(ab)}$	1.11	σ^2_{mg}	4.676	2.557	2.742
d_b	-0.16	$j_{(ba)}$	-2.49	σ^2_{pg}	0.014	0.552	3.927
h_a	-2.55	l	5.18	σ^2_e	3.402	3.402	3.402
h_b	-1.80	[d]	4.85	h^2_{mg}	0.578	0.393	0.272
h_a/d_a	15.93	[h]	2.43	h^2_{pg}	0.002	0.085	0.390
h_b/d_b	11.25			$\sigma^2_{mg} / \sigma^2_G$	0.997	0.822	0.411

d_a: additive effects of the first major gene; d_b: additive effects of the second major gene; h_a: dominant effects of the first major genes; h_b: dominant effects of the second major genes; h_a/d_a: dominance degree of the first major genes; h_b/d_b: dominance

degree of the second major genes; i: the effect of additive × additive between two major genes; j_{ab}: the effect of additive × dominant between two major genes; j_{ba}: the effect of dominant × additive between two major genes; l: the effect of dominant × dominant between two major genes; [d]: the additive effect of polygenes; [h]: the dominance effect of polygenes; σ_p^2: phenotypic variance; σ_{mg}^2: variance of major genes; σ_{pg}^2: variance of polygenes; σ_e^2: environmental variance; h_{mg}: inheritability of major genes; h_{pg}: inheritability of polygenes.

Previous study reported that oil content in rapeseed controlled only by additive effects (Engqvist et al., 1991), or predominance of the additive effects (Variath et al., 2009). QTL mapping also demonstrated additive effects to be the main factor but few epistatic effects were identified contributing to variation on oil content (Delourme et al., 2006, Jiang et al., 2014), and a substantial contribution was explained by additive × additive epistatic effects except additive effects (Zhao et al., 2005). However, dominance effects played an important role in controlling of the oil content and explained 46.94% of the total variance was reported recently (Wang et al., 2010). In this study, we revealed a genetic model with predominance of additive effects and epistatic effects on the inheritance of oil content by using generation mean analysis. The result was in consistence with most of the previous studies with additive effects as main factors controlling the inheritance of oil content. In contrast, the significant additive by additive and dominance by dominance effects also were detected in this study.

However, more details of gene action for oil content were revealed by using major gene plus polygene analysis which had been employed to identify the

gene action into major gene and polygene effects, respectively. The inheritance of certain traits in many crops had been studied using this method, such as the *cucumber mosaic virus* in chili pepper (Yao et al., 2013), flowering of chrysanthemum (Zhang et al., 2011), resistance to *Fusarium moniliforme* ear rot in maize (DengFeng et al., 2009), maize leaf spacing (Shilin et al., 2013), drought and stripe disease resistance in rice (Hu et al., 2011, Zheng et al., 2012) and coat color in Sesame (Zhang et al., 2013). In contrast with the generation mean analysis for oil content in this study, the predominance of additive gene effect resulted from polygenes but not major genes. On the other hand, the magnitude of negative dominance effect of major genes and positive dominance effect of polygenes was found to govern the inheritance of oil content, and the opposite directions of dominance effects resulted in no significant dominance effects which were detected by generation mean analysis.

Oil content in *B. napus* is generally determined as a character with high broad sense heritability with score as 83.3% to 90% (Jiang et al., 2014, Wang et al., 2010). In this study, we reported moderate heritability of major genes in BC_1 generations, whereas the lower heritability of major genes were detected in BC_2 and F_2 generations, respectively. In contrast, the heritability of polygenes accounted for more than half of the genetic variance in F_2 generation while very lower genetic variance could be explained by polygenes in BC_1 and BC_2 generation. The results indicated high efficiency of selecting for oil content in progenies of backcross rather than F_2 generation. In addition, selecting effectively in $F_{2:3}$ generation rather than F_2 generation had been suggested (Zhang et al., 2006).

4.5 Conclusion

In this study, the oil content in rapeseed was mainly controlled by additive effect and dominance effect of polygenes, as well as dominance effect and dominance × dominance epistatic effect of major genes. The magnitude of the dominance effects for polygenes and major genes are similar with opposite orientation, which maybe the reason of dominance effect is not significant for oil content with the generation mean analysis. In addition, the additive effect and dominance effect of polygenes are more important than major genes, which bring a challenge for improvement of oil content in rapeseed breeding. Moreover, the backcross is an effective method for high oil content breeding due to the higher determination of variance by major genes.

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CHAPTER V

A HIGH DENSITY DART-SEQUENCING BASED GENETIC LINKAGE MAP CONSTRUCTION IN RAPESEED

5.1 Abstract

Genotyping for 91 individual plants in BC_1 population of a 10-Zi006×10-4438 rapeseed cross by DArT-seq technology resulted in 9372 polymorphic markers including 5968 *in silico* DArT markers and 3404 SNP markers. Form those markers, high quality SNP were selected firstly for framework map construction. The other markers including *in silico* DArT markers, lower quality SNP markers and distorted markers were attached gradually into the map after genotyping errors were removed by SMOOTH software. The high-density genetic linkage map was finally constructed by online MST_{MAP} program containing 1614 discrete loci with 9212 markers and covered 3616.38cM. The average genetic distance between loci was 2.24cM. A genome accounted for 61.65% of loci and 65.73% of markers, the average genetic distance between loci was 2.22cM and 2.65cM on A and C genome, respectively. The segregation distortion was also analyzed in this study. In addition, 74.43%, 50.61% and 53.19% of *in silico* DArT markers and 88.33%, 70.88% and 70.82% of SNP markers were aligned with *B. napus* L., *B. rapa* L. and *B. oleracea* L. genome, respectively. This high-density map was useful for genetic studies, such as QTLs identification for interesting traits in rapeseed

5.2 Introduction

Linkage map based on molecular markers is important tool in rapeseed genetic analysis. This technique have been used for localization of genes underlying quantitative traits, marker assisted breeding and map based gene cloning. In the past, the genetic linkage map in rapeseed had been constructed by employing various types of molecular markers such as restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), sequence related amplified polymorphisms (SRAP), and single nucleotide polymorphism (SNP), and was further applied in rapeseed breeding programs (Delourme et al., 2013, Lombard et al., 2001, Qiu et al., 2006). However, the applications using these markers are time consuming and labor intensive due to the low-throughput system, although SSR and AFLP markers could be assayed on highly parallel genotyping platforms but they are expensive for genotyping. Thus, a cost and time efficient marker platform would be useful for whole genomic analysis.

Diversity Arrays Technology (DArT) is cost-effective genotyping technology detecting all types of DNA variation (SNP, indel, CNV, methylation). This technique was invented to overcome some limitations of other molecular marker technologies such as RFLP, AFLP and SSR (Kilian et al., 2012). The core of DArT technology is genome complexity reduction and provides a significant advantage via an intelligent selection of genome fraction corresponding predominantly to active genes (http://www.diversityarrays.com). This selection is achieved through the use of combination of restriction enzymes which separate low copy sequences from the repetitive fraction of the genome. For *Brassica*, the technology was optimized by

selecting the *Pst*I- *Mse*I method to reduce the $A^nA^nC^nC^n$ genomic complexity and produced SNP and Presence/Absence Markers (PAM) markers (Raman et al., 2014).

DArT technique have been employed widely on genetic linkage map construction for varied crops through the whole-genome profiling (Iorizzo et al., 2014, Milczarski et al., 2011, Schouten et al., 2012, Tomaszewski et al., 2012). In addition, high-density composite or consensus map had been constructed using DArT marker for genetic studies, such as segregation distortion analysis (Alheit et al., 2011, Li et al., 2010); Pan-genomic evolutionary studies (James et al., 2008); QTL identification (Cichy et al., 2014, Cui et al., 2014, Rahman et al., 2014). Moreover, DArT marker had been used to develop the first genetic map or first complete genetic map in some crops (Oliver et al., 2011, Yang et al., 2011). DArT technique as a high-throughput genotyping platform was utilized for high-density genetic map construction in rapeseed (Raman et al., 2014). Furthermore, the first *B. napus* consensus map was constructed based on the Diversity Arrays Technology (DArT) and non-DArT markers, this consensus map could be used to identify candidate genes underlying natural genetic variation for traits of interest (Raman et al., 2013).

However, selection of computer programs, as well as genotyping error removal is very important for high-density map construction based on high-throughput genotyping platform. Some available computer programs for linkage mapping are incapable of handling data set of several thousands of markers, and result in prohibitively long calculation times. On the other hand, even small frequencies of scoring error result in high rates of ordering ambiguities between markers within short genetic distances (Van Os et al., 2006). In this study, we used SMOOTH combined with JionMap v4 for genotyping error removal. SMOOTH was developed to remove

genotyping errors through calculation of the probability of a data point being a ‘‘singleton’’ on the basis of neighboring marker information. The observation of singletons depend on their context of flanking markers. Therefore, singletons are removed in an iterative process, singleton removal, reordering of markers, singleton removal, reordering, etc., thereby gradually relaxing the statistical threshold of singleton identification (van Os et al., 2005). The SMOOTH have always been combined with any of the mapping programs, such as RECORD-WIN, JionMap and MST_{MAP}, etc. Even the loss of a few percentages of the data though the genotyping error removal, however it is obviously less damaging to the map than having similar levels of genotyping errors (van Os et al., 2005). For high-density map construction, a novel online program named MST_{MAP} was used to cluster and order thousands of markers. This method is computationally very efficient and also gracefully handles missing observations and is capable of tolerating some genotyping errors. MST_{MAP} is freely available in the public domain at http://www.cs.ucr.edu/, yonghui/MST_{MAP}.html (Wu et al., 2008).

In this study, we reports a DArT-seq markers-based high-density genetic linkage map construction in rapeseed using BC_1 population which was derived from 10-Zi006 x 10-4438 cross. This map could be utilized for identification of QTLs associated with important traits in rapeseed, such as anthocyanin content; oil content, chlorophyll content and fatty acid components.

5.3 Materials and Methods

5.3.1 Mapping population

BC_1 population comprising 91 lines from 10-Zi006 × 10-4438 was used for

genetic linkage map construction. 10-Zi006 is an elite inbred line with purple-red leaf developing through an interspecific hybridization using Youyan 2 (*B. napus*) as male and Hong youcai (*B. campestris*) as female; 10-4438 with green leaf was developed by self-pollination of P6036-1.

5.3.2 DNA isolation

Young leaf tissue of individual plant in P_1, P_2, F_1 and BC_1 population was collected and ground to a fine powder using a Geno/Grinder™ 2000 (SPEX Sample Preparation, USA), a modified CTAB procedure (Cullings, 1992, Doyle et al., 1990) was then used for DNA extraction (Appendix Table 1).

5.3.3 Genotyping

The whole-genome profiling was performed using the DArT-Seq technique by Diversity Arrays Technology Pty Ltd (DArT P/L, Yarralumla, ACT, Australia). The DArT-Seq technology was optimized for *Brassica* by selecting the *Pst*I- *Mse*I method to reduce the $A^nA^nC^nC^n$ genomic complexity. DNA samples were processed in digestion/ligation reactions with two different adaptors corresponding to two different Restriction Enzyme (RE) overhangs. Only *Pst*I-*Mse*I fragments were effectively amplified in 30 rounds of PCR. After PCR, equimolar amounts of amplification products from each sample of the 96-well microtiter plate were multiplexed and applied to c-Bot (Illumina) bridge PCR followed by sequencing on Illumina Hiseq2000. All amplicons were sequenced in a single lane. The sequencing (single read) was run for 77 cycles. Sequences generated from each lane were processed using proprietary DArT analytical pipelines. In the primary pipeline, the FASTQ files were first processed to filter away poor quality sequences; more stringent selection criteria (≧Phred pass score of 30) were applied to the barcode region than to the rest of the

sequence. As a result, the assignments of the sequences to specific samples carried in the barcode split step were very reliable. Approximately 2,000,000 sequences per barcode/sample were identified and used in marker calling. Finally, identical sequences were collapsed into fastqcall files. These files were used in the secondary pipeline for DArT P/L's proprietary SNP and Presence/Absence Markers (PAM) calling algorithms (DArTsoft-seq) (Raman et al., 2014). The scores of SNP and *in silico* DArT markers were provided by DArT P/L with two types as present=1 vs. absent=0. The sequences of DArT-Seq markers were aligned to *B. rapa*, *B. oleracea* and *B. napus* reference genome sequence.

5.3.4 The framework map construction (map 1)

SNP markers were renamed with adding "SNP" ahead of the clone ID, the name of *in silico* DArTs markers were with clone ID only. The SNP markers with high quality were selected for genetic linkage framework map construction based on two quality control parameters, AvgCount≥7.0 and RepAvg≥0.989. Furthermore, The SNP and *in silico* DArTs markers with a broader selection were gradually attached to the framework map. The broader selection parameters for the SNP were PIC≥0.3, AvgCount≥4, RepAvg ≥ 0.975 and CallRate ≥0.9. *In silico* DArTs markers were selected based on Reproducibility≥0.94, Qpm≥1.50, AvgReadDepth ≥ 4.0 and CallRate ≥0.855.

A Perl script was written for grouping loci with identical segregation patterns disregarding unknown scores. The marker with the highest call rate (percentage of scored individual) was selected as a representative for each group. High quality SNP markers were clustered into linkage groups using JionMap v4.0 (Van Ooijen, 2006) with LOD score of ≥3.0 after filtering distorted markers with

p=0.05. The linkage groups were assigned using the markers information which were aligned with *B. napus* genome. The software SMOOTH was then used for genotyping error removal, which was applied in conjunction with JionMap v4.0 in maximum likelihood mapping algorithm by cyclically reiterating the process of marker ordering and singleton removal (van Os et al., 2005). Pairwise recombination values among all markers were calculated for each linkage group by Joinmap v.4. The markers with zero recombination among them were considered to belong to the same genetic bin. The marker with the least missing data points was chosen to represent each bin for genetic mapping. The markers, including the markers that represent each bin, were mapped using JionMap4.0 with regression mapping algorithm. The map order optimization parameters had been used as follow: the linkages with a recombination frequency smaller than 0.4 and a LOD larger than 1.00, the goodness-of-fit jump threshold for removal of loci 5.00, number of added loci after which to perform a ripple 1. The genetic distances were calculated by using Kosambi's mapping function.

5.3.5 High-density map construction

The polymorphic SNP and *in silico* DArTs markers excluding the distorted markers (*p*=0.01) were selected with broad selection parameters to construct high-density genetic linkage framework map 2. The genotyping error removal was performed by SMOOTH software under the certain marker order which were calculated by JionMap v4.0 at maximum likelihood mapping algorithm with the "fixed order" function. In each cycle of genotyping error removal, the marker order in map 1 was used for "fixed order". Other steps, such as grouping and regression mapping followed the section 4.3.3.

A high-density genetic linkage map (map 3) including all polymorphic SNP and *in silico* DArTs markers were constructed by using MST_{MAP} online program. The markers were clustered into genetic linkage group by using MST_{MAP} online with grouping LOD criteria as 10. The genotyping error removal were also performed by SMOOTH software in combined with JionMap v4.0. The "fixed order" function in JionMap v4.0 with maximum likelihood mapping algorithm were applied to make sure the same marker order with the framework map 2. After cleaning the data, the MST_{MAP} online was used to construct the high-density map with the map order optimization parameters: no mapping size threshold with 2, no mapping distance threshold with 15 centi-morgans, no mapping missing threshold with 25%, and try to detect genotyping errors. The genetic distances were calculated by using Kosambi's mapping function.

5.4 Result and Discussion

5.4.1 Genotyping by DArT-Seq technology

The total of 9372 polymorphic markers were obtained by DArT-seq, and 5969 out of which were *in silico* DArT markers accounting for 63.69% of total polymorphic markers. In contrast, the number of SNP markers (3403) accounted for 36.31% of total markers. The average of CallRate, AvgReadDepth, Qpmr and Reproducibility of *in silico* DArT markers are 92.20%, 10.83, 2.10 and 99.69%, respectively. The average of CallRate, AvgCount, AvgPIC and RepAvg of SNP markers are 99.90%, 9.90, 48.97% and 99.48%, respectively. There were 4443, 3379 and 3175 of *in silico* DArT markers aligned with *B. napus* L., *B. rapa* L. and *B. oleracea* L. genome, accounted for 74.43%, 50.61% and 53.19% of the total *in silico*

DArT markers, respectively. There were 3006, 2412 and 2410 of SNP markers aligned with *B. napus* L., *B. rapa* L. and *B. oleracea* L. genome, accounted for 88.33%, 70.88% and 70.82% of the total SNP markers, respectively. For the SNP markers, 1452 (43.24%) were transversions and 1906 (56.76%) were transitions (Appendix Table 6).

5.4.2 Construction of genetic linkage framework map 1

Based on the selection parameters (AvgCount≥7.0 and RepAvg≥0.989), 2052 of polymorphic SNP markers were employed for genetic linkage framework map 1 construction, and in which the average of CallRate, OneRatio, PIC, AvgCount and RepAvg was 0.99, 0.54, 0.49, 12.23 and 0.99, respectively. 234 (11.4%) out of the 2052 SNP markers showed segregation distortion, and 1818 remaining SNP markers were grouped by JionMap v4.0 with recombination frequency as 0.23. In total, 19 linkage groups (number of markers over 10) were identified, in which the A2 chromosome was absent while the C9 chromosome was divided into two linkage groups (Table 5.1). The genetic linkage framework map 1 of BC_1 population (10-10-Zi006 × 10-4438) contained 1050 loci with 1774 markers. The markers with the same locus was regarded as a "bin" and 333 bins were detected and presented 1084 markers within the total 19 linkage groups. The map distance covered 1479.04 cM with average 1.41cM across all the chromosomes. A genome account for 60.57% of the loci and 65.85% of markers which were denser than B genome. However, the A3, A6 and C3 were genetic linkage groups with richer marker loci than other groups (Table 5.1, Appendix Figure 1).

Table 5.1 The number of markers, bins and marker coverage within the linkage groups of the genetic map `1 of the BC_1 population in *B. napus* based on the high-quality SNP markers.

Chromosome	Linkage group [a]	No. Loci [b]	No. total mapped marker [c]	Coverage (cM)	Average Marker Density/chromosome (cM)
A1	A1	52 (20)	103 (71)	88.29	1.70
A3	A3	112 (38)	190 (115)	133.66	1.19
A4	A4	43 (19)	88 (64)	68.20	1.59
A5	A5	79 (30)	167 (118)	71.48	0.90
A6	A6	101 (28)	171 (98)	51.05	0.51
A7	A7	69 (26)	120 (77)	100.94	1.46
A8	A8	59 (21)	111 (73)	58.06	0.98
A9	A9	60 (15)	88 (44)	99.66	1.66
A10	A10	61 (26)	130 (95)	62.90	1.03
Subtotal		636 (223)	1168 (755)	734.24	1.15
C1	C1	38 (10)	53 (25)	103.15	2.71
C2	C2	51 (15)	91 (55)	36.94	0.72
C3	C3	94 (28)	114 (75)	147.29	1.57
C4	C4	51 (14)	72 (35)	101.87	2.00
C5	C5	31 (6)	40 (15)	102.58	3.31
C6	C6	10 (1)	11 (2)	10.35	1.04
C7	C7	40 (9)	54 (23)	86.70	2.17
C8	C8	49 (13)	79 (43)	92.66	1.89
C9	C9-1	41 (11)	69 (39)	57.68	1.41
	C9-2	9 (3)	23 (17)	5.57	0.62
Subtotal		414 (110)	606 (329)	744.80	1.80

Table 5.1 (Continued) The number of markers, bins and marker coverage within the linkage groups of the genetic map `1 of the BC_1 population in *B. napus* based on the high-quality SNP markers.

Chromosome	Linkage group [a]	No. Loci [b]	No. total mapped marker [c]	Coverage (cM)	Average Marker Density/chromosome (cM)
Total		1050 (333)	1774 (1084)	1479.04	1.41
Mean		55.26 (17.53)	93.37 (57.05)	77.85	1.41

[a] The linkage groups were assigned in accordance with the alignment with *B. napus* genome. [b] The numerals shown in the bracket are the number of loci present as bins in the linkage groups. [c] The numerals shown in the bracket are the total number of markers integrated into bins in the linkage groups

In order to avoid a contradictory placement of loci that occurred occasionally when attached any distorted markers or other lower quality markers, individual maps were recalculated by setting individual loci at "fixed order" (Holtgräwe et al., 2014, Tondelli et al., 2014). So, the purpose of the construction for genetic linkage framework map 1 by high quality SNP markers excluding the segregation distortion was to accurately identify the markers orders on certain chromosomes. However, A2 chromosome was absent in this framework genetic linkage map due to the all markers were segregation distortion on the chromosome A2 (see the analysis of section 5.4.3.2).

5.4.3 Construction of high-density genetic linkage map

According to broad selection, the total of 5968 *in silico* DArT markers and

3404 SNP markers were selected for high-density genetic linkage map construction. The allelic distortion analysis shown 578 and 427 of *in silico* DArT markers, 283 and 174 of SNP markers were segregation distortion with *p*-value at 0.05 and 0.01, respectively. Total 8771 of SNP and *in silico* DArT markers including the markers with distortion at p-value at 0.05 were used for high-density genetic linkage framework map 2 construction with JionMap v4.0, and the total of the 9372 SNP and *in silico* DArT markers were used for high-density map 3 construction with the online MST_{MAP} program.

5.4.3.1 Construction of high-density framework map 2

A Perl script analysis resulted in 3440 unique segregation patterns (bins) from the total 8771 of SNP and *in silico* DArT markers. Representative markers with highest call rate in those unique segregation patterns were selected for genetic linkage map construction. Based on the recombination frequency of 0.23, 3435 out of 3440 bins could be clustered to 18 linkage groups. Subsequently, two linkage groups were divided into two linkage groups, respectively. According to the align information with the *B. napus* genome, total 20 genetic linkage groups were assigned to 19 chromosomes, however, two groups were assigned into the C9 chromosome.

The markers in each linkage groups were ordered firstly by JionMap v4.0, and the “fixed order” function in maximum likelihood mapping algorithm were applied to make sure the same order with the framework map 1 which was constructed by using only high quality SNP markers. SMOOTH software was then used for genotyping error removal under the certain order. After 4 to 14 cycles, the suspicious genotyping in each groups were removed and transferred into “missing data” which could be resulted in “super bin”. Genetic linkage map was identified with

20 linkage groups on 19 chromosomes, in which the 2235 loci including 1632 of SNP and 603 of *in silico* DArT markers represented total 8732 markers, and 7593 markers that had been assigned into 1070 bins. The map distance covered 1721.65 cM with average 0.77cM across all the chromosomes. Genome A account for 59.64% of marker loci and 62.09% of markers, and both the marker loci and number of markers were dense than genome B. However, A3, A6 and C3 with richer marker loci were dense than other groups (Table 5.2, Appendix Figure 2).

Table 5.2 The number of markers, bins and marker coverage within the linkage groups of the genetic map 2 of the BC_1 population in *B. napus* based on SNP and *in silico* DArT markers.

Chromosome	Linkage group [a]	No. Loci [b]	No. total mapped marker [c]	Coverage (cM)	Average Marker Density (cM)
A1	A1	149 (50)	504 (405)	105.98	0.71
A2	A2	8 (3)	20(15)	9.75	1.22
A3	A3	189 (106)	868(785)	134.41	0.71
A4	A4	109 (58)	505 (454)	70.99	0.65
A5	A5	162 (68)	788 (720)	79.62	0.49
A6	A6	191 (86)	757 (652)	116.83	0.61
A7	A7	144 (75)	508 (439)	101.89	0.71
A8	A8	136 (64)	552 (480)	98.99	0.73
A9	A9	123 (57)	351 (285)	66.41	0.54
A10	A10	122 (68)	569 (515)	67.89	0.56
Subtotal		1333 (635)	5422 (4750)	852.76	0.64
C1	C1	65 (30)	222 (187)	95.88	1.48
C2	C2	113 (57)	558 (502)	50.49	0.45

Table 5.2 (Continued) The number of markers, bins and marker coverage within the linkage groups of the genetic map 2 of the BC_1 population in *B. napus* based on SNP and *in silico* DArT markers.

Chromosome	Linkage group [a]	No. Loci [b]	No. total mapped marker [c]	Coverage (cM)	Average Marker Density (cM)
C3	C3	187 (89)	634 (537)	136.88	0.73
C4	C4	125 (60)	373 (308)	110.14	0.88
C5	C5	72 (33)	198 (159)	106.79	1.48
C6	C6	60 (29)	195 (164)	92.80	1.55
C7	C7	85 (39)	204 (158)	107.85	1.27
C8	C8	106 (54)	423 (371)	99.40	0.94
C9	C9-1	69 (36)	353 (319)	60.78	0.88
	C9-2	20 (8)	150 (138)	7.89	0.39
Subtotal		902 (435)	3310 (2843)	868.89	0.96
Total		2235 (1070)	8732 (7593)	1721.65	0.77
Mean		111.75 (53.5)	436.60 (379.65)	86.08	0.77

[a] The linkage groups were assigned in accordance with the alignment with *B. napus* genome. [b] The numerals shown in the bracket are the number of loci present as bins in the linkage groups. [c] The numerals shown in the bracket are the total number of markers integrated into bins in the linkage groups

5.4.3.2 Construction of high-density map 3

The total of the 9372 SNP and *in silico* DArT markers were initially grouped with MST_{MAP} online at LOD=10 (http://138.23.178.42/MST_{MAP}/). There were 59 of linkage groups were clustered by MST_{MAP} online, in which the 16 of

linkage groups with number of marker range from 154 to 978, and the number of the bin more than 10 were selected for map construction. Four linkage groups subsequently were divided into 9 linkage groups by JionMap v4.0 under the recombination frequency with 0.14 or 0.15. Twenty one linkage groups were finally assigned into 19 chromosomes according to the markers information that had been aligned with *B. napus* genome. The A9 and C9 chromosome contained 2 linkage groups, respectively.

The JionMap v4.0 with the "fixed order" function in maximum likelihood mapping algorithm were combined with the software SMOOTH to remove genotyping errors. The number of 4125 genotyping error were removed and been translated into missing data (Table 5.3).

The genetic linkage map was constructed by online MST_{MAP} program after genotyping error removal, containing 1614 discrete loci with 9212 markers and covered 3616.38cM. The average genetic distance between loci was 2.24cM. The A genome accounted for 61.65% of loci and 65.73 of markers. Average genetic distance between loci on A genome was 2.22cM while 2.65cM on B genome (Table 5.4).

Table 5.3 The number of error genotyping were detected by SMOOTH software.

Linkage group	The No. of missing data before smooth [a]	The No. of missing data after smooth [b]	The No. of difference
A1	2618	2943	325
A2	1980	2107	127
A3	2923	3251	328
A4	1940	2121	181
A5	3097	3465	368
A6	2675	3047	372
A7	1731	1931	200
A8	2048	2294	246
A9-1	1239	1490	251
A9-2	746	826	80
A10	1801	2022	221
C1	579	643	64
C2	1991	2240	249
C3	2233	2527	294
C4	1240	1462	222
C5	742	831	89
C6	793	882	89
C7	508	606	98
C8	1579	1749	170
C9-1	645	735	90
C9-2	626	687	61
Total	33734	37859	4125

[a] The no. of missing data were detected Diversity Arrays Technology Pty Ltd.

[b] The error genotyping data had been translated to missing data, the number of missing data after smooth including the error genotyping removal detected by and detected by SMOOTH software.

Table 5.4 The number of markers, bins and marker coverage within the linkage groups of the genetic map of the BC_1 population in B. napus based on SNP and *in silico* DArT markers.

Chromosome	**Linkage group** [a]	**No. Loci**	**No. total mapped marker**	**Coverage (cM)**	**Average Marker Density (cM)**
A1	A1	116	636	289.48	2.50
A2	A2	39	340	92.67	2.38
A3	A3	148	867	305.40	2.06
A4	A4	78	506	144.80	1.86
A5	A5	108	790	200.72	1.86
A6	A6	127	763	265.89	2.09
A7	A7	97	509	207.52	2.14
A8	A8	87	552	177.62	2.04
A9	A9-1	89	350	191.86	2.16
	A9-2	20	173	64.63	3.23
A10	A10	86	569	184.05	2.14
	Subtotal	995	6055	2124.641	2.22
C1	C1	33	165	85.89	2.60
C2	C2	102	586	212.06	2.08
C3	C3	127	637	237.33	1.87

Table 5.4 (Continued) The number of markers, bins and marker coverage within the linkage groups of the genetic map of the BC_1 population in B. napus based on SNP and *in silico* DArT markers.

Chromosome	Linkage group [a]	No. Loci	No. total mapped marker	Coverage (cM)	Average Marker Density (cM)
C4	C4	89	396	241.05	2.71
C5	C5	53	198	147.73	2.79
C6	C6	40	192	134.07	3.35
C7	C7	48	200	99.55	2.07
C8	C8	80	425	209.92	2.62
C9	C9-1	35	204	72.01	2.06
	C9-2	12	154	52.14	4.35
Subtotal		619	3157	1491.739	2.65
Total		1614	9212	3616.38	2.24

[a] The linkage groups were assigned in accordance with the alignment with *B. napus* genome

The segregation distortion were observed for on all of the linkage groups except C1 and C9-1 (Table 5.5). However, the linkage groups A1, A2, C4 and C6 accounted for most of the distorted markers. On A2 linkage group, the total of the 340 markers showed segregation distortion and accounted for 100% of the markers in this linkage group, it also accounted for 44.33% of the total distorted markers. A1 linkage group with 130 distorted markers account for 16.95% of the total distorted markers. However, there were no distorted marker on linkage group C1 and C9-1.

Table 5.5 The number and the proportion of segregation distortion markers in each linkage group.

Linkage group	No. of total markers	No. of distorted markers ($p<0.05$)	% of markers on linkage group	% of total distorted markers
A1	636	130	20.44	16.95
A2	340	340	100.00	44.33
A3	867	10	1.15	1.30
A4	506	14	2.77	1.83
A5	790	1	0.13	0.13
A6	763	26	3.41	3.39
A7	509	7	1.38	0.91
A8	552	4	0.72	0.52
A9-1	350	1	0.29	0.13
A9-2	173	25	14.45	3.26
A10	569	15	2.64	1.96
C1	165	0	0.00	0.00
C2	586	18	3.07	2.35
C3	637	15	2.35	1.96
C4	396	68	17.17	8.87
C5	198	6	3.03	0.78
C6	192	68	35.42	8.87
C7	200	4	2.00	0.52
C8	425	11	2.59	1.43
C9-1	204	0	0.00	0.00
C9-2	154	4	2.60	0.52
Total	9212	767	-	-

In this study, three genetic linkage maps had been constructed. However, the final high-density map (map 3) constructed by using online MST_{MAP} program was employed for the further genetic analysis. The first genetic linkage framework map 1 constructed by using only high-quality SNP marker provided an accurate marker orders in each linkage group, which was useful on fixing the markers order when other lower quality or distorted markers attached in this map. The map 2 constructed based on fixing the markers order of the map 1, and more polymorphic markers were selected. Comparison with map 1 and map 2, more number of marker loci and more shorter loci interval was detected in the map 2, however the map length is moderate larger than the map 1. The map 3 was constructed by attaching all the polymorphic markers including the distorted markers, and fixing the markers order according to the map 2. However, the map length in map 3 was larger and the loci interval was higher than map 2. The map length in map 1 (1479.04 cM) and map 2 (1721.65 cM) were comparable with the previous maps of *B. napus* which ranged from 1196 cM to 2672 cM (Delourme et al., 2013, Raman et al., 2014, Zhao et al., 2005). However, the genetic linkage map with map length of 3628.87 cM in *B. napus* had been constructed for genome-wide delineation of natural variation of pod shatter resistance (Raman et al., 2014), which was accordance with map 3 with map length 3616.38 cM. As we know that genotyping errors could cause increases in genetic map sizes (Cartwright et al., 2007), however the SMOOTH software, which can remove genotyping errors from genetic linkage data during the mapping process (van Os et al., 2005), was employed for all map construction. We considered that the presence of distorted markers resulted in larger map size in map 3, those because the distorted markers could affect recombination frequency between markers and skew the genetic

distances between markers (Raman et al., 2014). 10-Zi006 as one of the parent in the mapping population, was developed from interspecific hybridization, which may be the reason that resulted in segregation distortion in mapping population. The segregation distortion had been supported by selection of gametophytes or sporophytes, chromosomal rearrangements which affect synapses in meiosis, genetic interaction among loci, nonhomologous recombination, gene conversation and/or transposition (Alheit et al., 2011) .

However, the marker order is more important than estimated map distances. An accurate marker order is indispensable for further application of the map, such as map based cloning (van Os et al., 2005). In addition, the order for co-segregate markers is indeterminate in the map construction. Therefore the construction of map 3, which was focused on the accurate marker order and genotyping error removal, was useful for further application of genetic studies on rapeseed.

5.5 Conclusion

In this study, the parents used for mapping population developing differed on genetic base that resulted in many polymorphic markers by DArT-seq technology. However, the one of the parent 10-Zi006 had been developed by distant hybridization, which maybe result in the chromosomal rearrangements, gene conversation and/or transposition, etc. We can reasonably infer that this maybe the reason of resulting in segregation distortion, especially all the distorted markers on A2 linkage group. Furthermore, the markers align with the *B. napus* L. genome would may be affected under this reason. However, 74.43% of *in silico* DArT markers and 88.33% of SNP

markers were aligned with *B. napus* L. genome, and the process of the map construction was emphasized on the marker order by genotyping error removal and fixing the order. Therefore, this high-density map of 10-Zi006 × 10-4438 was useful for genetic studies, such as QTLs identification, gene cloning for interesting traits in rapeseed.

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CHAPTER VI

IDENTIFICATION OF QTL FOR ANTHOCYANIN, CHLOROPHYLL AND OIL CONTENT IN RAPESEED

6.1 Abstract

The seed oil, leaf anthocyanin and chlorophyll contents are the important traits related to yield and economic benefits in rapeseed (*B. napus* L.). Understanding the genetic basis of those complex quantitative traits may provide the potentiality in breeding for improve seed oil content and energy conversion efficiencies, even on resistance to drought and cold temperature. Here, the genetic analysis for the traits were explored using a novel genotyping-by-sequencing approach DArT-Seq. A total of 33 significant QTL were identified. Eleven QTL associated with seed oil content were detected on A03, A05, A06, A07, A10, C05 and C09 chromosomes explaining individually 1.99-18.30% of phenotypic variation, 14 QTL controlling the leaf anthocyanin content were identified on A03, A04, A05, A06, C02 and C04 chromosomes explaining 9.17-19.13% of phenotypic variation and 8 QTL for leaf chlorophyll content were mapped on A01, A03, A06, A07, C01, C03 and C04 chromosomes explaining 4.45-13.07% of phenotypic variation. Most of the QTL region was well matched on the physical map with the intervals of 0.02 to 14.54 Mbp. Our results shown some shorter QTL regions physically with a few linkage marker indicating possibility to detect candidate genes controlling the traits by analysis of the

genomic region. We also speculate that some markers tightly linked with the QTL themselves maybe are the candidate gene. These findings enriched knowledge on the genetic basis for the study traits and would be a potential in aspect of map-based cloning as well as marker-assisted breeding.

6.2 Introduction

Rapeseed (*Brassica napus* L., AACC, 2n = 38) is the third largest oilseed crop after oil palm and soybean and also considered as new resource of biodiesel. With the increasing demand for rapeseed oil, the studies on the genetic control of oil content has been emphasized as an important objective in rapeseed breeding (Wang, 2004).

Oil content in rapeseed is a complex quantitative trait controlled by multiple genes and influenced by environmental factors (Si et al., 2003, Zhao, J. Y. et al., 2005). This traits varies quantitatively among genermplasm of rapeseed due to the complex regulation of multiple genes on metabolism of seed oil (Barker et al., 2007). The genetic control of oil content in rapeseed were reported to be controlled by additive effect (Delourme et al., 2006, Shen et al., 2005, Variath et al., 2009, Zhang et al., 2006), additive-dominant-epistasis genetic model (Wang et al., 2010) and one additive major gene plus additive and dominance polygenes (Zhang et al., 2006). However, better understanding of the determinants of this trait is very important in breeding *B. napus* for improve oil content. QTL mapping is an effective way to dissect the genetic mechanisms of complex quantitative traits, and many QTL had been identified for oil content in various crops, such as wheat (Moore et al., 2015), peanut (Pandey et al., 2014, Varshney, 2015), G. soja (Wang, Wubin et al., 2014) and soybean (Akond et al., 2014, Wang et al., 2015). In *B. napus*, the earlier QTL controlling seed oil content

were detected to be closely associated with variation of erucic acid content (Ecke et al., 1995). Subsequently, different population were developed to map QTL associated with oil content in rapeseed, such as DH population (Chen et al., 2010, Delourme et al., 2006, Zhao, J. et al., 2005) and RILs population (Yan et al., 2009). With the effort of investigation on oil content in *B. napus*, a number of QTL were identified and most of those QTL can explain less than 20% of phenotypic variation (Wang et al., 2013). In order to compare the QTL detected on different population, the consensus genetic linkage map were constructed for detecting the consensus QTL based on the same markers (Jiang et al., 2014, Lombard et al., 2001, Raman et al., 2013). Moreover, most of the consensus QTL controlling the seed oil content in rapeseed were located on A1, A2, A3, A5, A6, A7, A8, A9, A10, C1, C2, C3, C5, C6, C8 and C9 linkage groups (Jiang et al., 2014, Wang et al., 2013).

Anthocyanin, a type of flavonoid pigments, is commonly responsible for orange red to violet blue color in plant tissues (Tanaka et al., 2008). It have been shown to be beneficial to human health that reduce certain cancers, coronary heart diseases, oxidative stress and other age-related diseases (Shin et al., 2006, Williams et al., 2008). Moreover, anthocyanin can protect plants against various biotic and abiotic stresses (Dodd et al., 1998, Klaper et al., 1996). The biosynthesis of anthocyanin was regulated by transcription factors, such as MYC, MYB, WD40-like protein, WRKY, MADS and TFIIIA-like protein (Cichy et al., 2014).

Identification of QTL related to genomic regions is essential for the understanding of the quantitative genetic control of anthocyanin. In *B. rapa* L. One QTL for seedling anthocyanin was identified within the A. *thaliana Transparent Testa12 (AtTT12)* ortholog (Rahman et al., 2014). A novel locus *Anp* was found

independent of *an1* anthocyanin-regulating factor (Hayashi et al., 2010, Mol et al., 1998). In addition, *BrPur*, a single dominant gene for purple leaf was mapped to linkage group A03, and subsequently was defined a genetic interval of 0.6 cM and a genomic region of 54.87 kb. For *B. napus* L., the studies on genetic control of anthocyanin mainly focused on the seed colour (Jiang et al., 2013, Lu et al., 2012). However, more emphasis had been focused on the leaf anthocyanin due to its antioxidant-related properties (Zhao et al., 2013). Because of the limitations of *B. napus* as a source of anthocyanin, two transcription factor genes of the anthocyanin biosynthesis pathway, *Del* and *Ros1*, were cloned and expressed heterologously in *B. napus*, which could increase the anthocyanin content ten-fold and the antioxidant activity up to three-fold. Therefore, the development of rapeseed line with high anthocyanin is useful for further studies and applications of antioxidant-related properties. We have developed rapeseed inbred line "Zigan" with purple red leaf by interspecific hybridization. This obvious color started at cotyledons stage, and change to green color differed from line to line. The utilization of purple red leaf in rapeseed had been recommended as an effective way to identify the false hybrid of F_1 (Wang, T. Q. et al., 2007, Wu et al., 2007). Moreover, the rapeseed cultivars with anthocyanin in leaf are resistant to drought and frost (Chalker‐Scott, 1999, Solecka et al., 1999). Thus, the one of the aim in this study is to identify QTL that control the anthocyanin content in rapeseed leaf.

Chlorophyll plays an important role in photosynthesis for plant growth, such as light-harvesting and energy transduction. The biochemical and genetic approaches were applied to understand the inheritance and biosynthesis of chlorophyll (Oster et al., 2000, Zhao et al., 2014). Nowadays, all the genes for the chlorophyll biosynthetic

steps in angiosperm plants have been identified (Beale, 2005), and the whole pathway of chlorophyll biosynthesis can be subdivided into four parts including the formation of 5-aminolevulinic acid, the formation of a pyrrole ring porphobilinogen and the synthesis of the first closed tetrapyrrole, the formation of protoporphyrin IX (Proto IX) and the formation chlorophyll from Proto IX (Tripathy et al., 2012). In *B. campestris* L., the studies of QTL identification for chlorophyll content or chlorophyll deficient-related genes were conducted. Ten QTL accounting for 7-17% of the phenotypic variation were detected in the Chinese cabbage(Ge et al., 2012); Four QTL, *CHL1*, *CHL2*, *CHL3* and *CHL4* were identified for *B. rapa* on A1, A6, A9 and A10 linkage groups. In addition, *BnChd1-1*, a locus involved in chlorophyll biosynthesis in *B. napus* was mapped to a region of A01 and identified the homologue of Bra040517 as candidate gene for *BnChd1-1*.

However, the 10-Zi006, plant material in this study, is a rapeseed inbred line with purple-red leaf existing in the whole seeding stage. The genetic control of the chlorophyll seemed to be important especially in purple-red leaf due to the possible effects of anthocyanin were confounded by a decrease in photochemical efficiency with chlorophyll loss in senescing leaf of *Cornus sanguinea* and *Parthenocissus quinquefolia* (Manetas et al., 2011). Moreover, a significant decrease of photosynthetic pigments simultaneous with an increase of anthocyanin was measured in *poinsettia bract* development (Slatnar et al., 2013). Therefore, another aim in this study is to detect the QTL that control the chlorophyll content in the purple-red leaf of rapeseed.

6.3 Materials and Methods

6.3.1 Plant materials

BC_1 population comprising 91 plants from (10-Zi006 × 10-4438)/ 10-4438 was used for genetic linkage map construction. 10-Zi006 is an elite inbred line with purple-red leaf developing through interspecific hybridization using Youyan 2 (*B. napus*) as male and Hong youcai (*B. campestris*) as female. 10-4438 with green leaf was developed by self-pollination of P6036-1.

6.3.2 Measurement of anthocyanin content

The anthocyanin content of individual plant in each generations were measured by photometric method (Mehrtens et al., 2005) at the seedling stage (with 6 to 7 leaf). The top expanded leaf were extracted at the same day for all plants in experiment. One milliliter of acidic methanol (1% HCl, w/v) was added to 300 mg of fresh leaf. The samples were incubated for 18 h at room temperature under moderate shaking, then sedimented by centrifugation (12710 g at room temperature for 1 minute) and 400 µl of the supernatant was added to 600 µl of acidic methanol. Absorption of the extracts at 530 and 657 nm wavelength had been determined by a spectrophotometer (UV-7504, Shanghai, China). Quantification of anthocyanin was performed using the following equation:

$$Q_{\text{anthocyanin}} = (A_{530} - 0.25 \times A_{657}) \times M^{-1}$$

Where Q anthocyanin is the amount of anthocyanin, A_{530} and A_{657} are the absorption at the indicated wavelengths and M is the weight of the rapeseed leaf used for extraction.

6.3.3 Measurement of chlorophyll content

Total chlorophyll content were determined by SPAD Chlorophyll Meter Reading (SCMR) in the same growth stage when the measurement of anthocyanin content. Each top expanded leaf were measured five times in different parts to monitor the chlorophyll status. SCMR was recorded using a chlorophyll content meter (Minolta SPAD-502 plus, Osaka, Japan)

6.3.4 Measurement of Oil content

The oil content in the seed extraction by using Soxhlet extraction methods. Soxhlet extraction was carried out using petroleum ether as the solvent. Rapeseed powder (2.0 g) with mesh size of 60 was weighed (A). The sample was then roasted for 3 h at 105 ℃ in hot air oven and cooled down for 30 min. The resulting sample was then weighed (B). The rapeseed oil was then extracted by a Soxhlet extractor. The Soxhlet extraction process took about 6 h, with an extraction temperature of 100℃. After reaction, the filter bag was baked for 30 min and weighed (C). Oil extraction rate was calculated, and oil percentage (w/w) was obtained with respect to the total oil present in rapeseed using follow equation (Martínez et al., 2008).

$$Y=(B - C) / A \times 100$$

6.3.5 DNA extraction

Young leaf tissue of individual plant in P_1, P_2, F_1 and BC_1 population was collected and ground to a fine powder using a Geno/Grinder™ 2000 (SPEX Sample Preparation, USA), a modified CTAB procedure (Cullings, 1992, Doyle et al., 1990) was used for DNA extraction (Appendix Table 1).

6.3.6 Genotyping

The whole-genome profiling was performed using the DArT-Seq technique by Diversity Arrays Technology Pty Ltd (DArT P/L, Yarralumla , ACT, Australia) (section 5.3.3).

6.3.7 The high-density genetic linkage map construction

The construction of high-density genetic linkage map using online MST_{MAP} program (see section 5.3.4, 5.3.5)

6.3.8 QTL mapping

WinQTL Cartographer 2.5 software with composite interval mapping (Wang, S. et al., 2007) was used to detect QTL controlling the anthocyanin, chlorophyll and oil content. Permutation test with 1000 times was performed to obtain a critical logarithm of the odds (LOD) threshold at significance level of a = 0.05 (Churchill et al., 1994, Doerge et al., 1996). The walking speed, window size, and number of control markers were set to 1 cM, 10 cM and 5, respectively. The confidence interval was set to 95%. QTL were named based on the standard nomenclature (McCouch et al., 1997).

6.4 Result and Discussion

6.4.1 Phenotypic variation for anthocyanin, chlorophyll and oil content

A continuously distribution for oil, anthocyanin and chlorophyll contents were investigated with 91 individual in BC_1 population of 10-Zi006/ 10-4438 (Figure 6.1) indicating that the seed oil content, leaf anthocyanin and chlorophyll contents in rapeseed were quantitative traits. In addition, multiple peaks were presented on frequency distribution for those studied traits. However, skewed distribution with the

value of Skewness of -2.82 (p = 0.0049) and 3.22 (p = 0.0013) for oil and anthocyanin content were found in the BC_1 population, respectively. On the other hand, the value of Skewness with -0.73 (p = 0.4683) indicated that chlorophyll content in rapeseed leaf was followed the normal distribution.

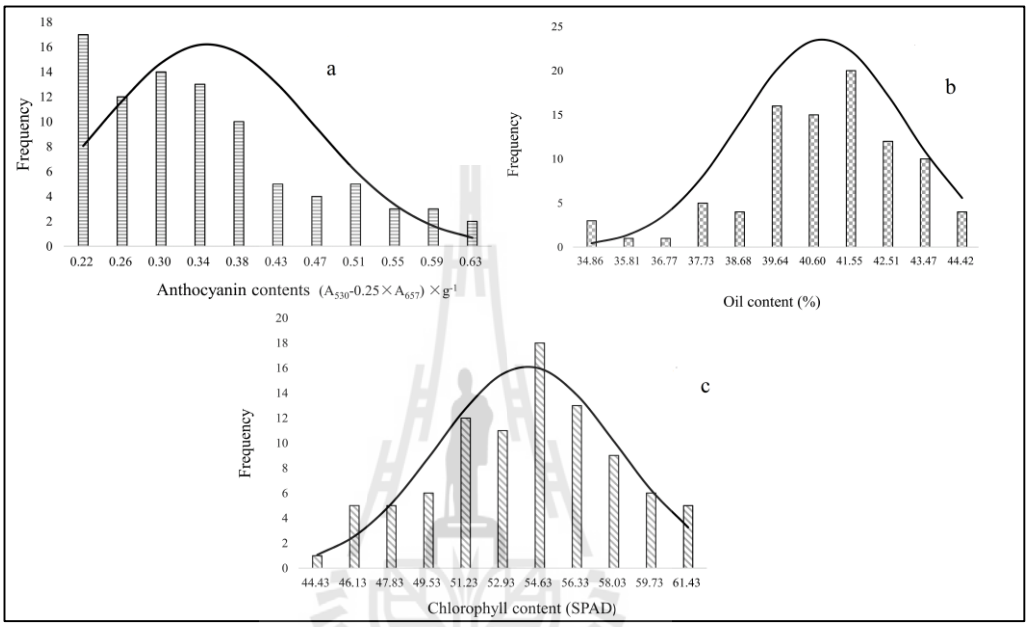

Figure 6.1 The frequency distribution of oil (a), anthocyanin (b) and chlorophyll content (c) in BC_1 population of 10-4438 × 10- Zi006

6.4.2 Mapping QTL controlling the seed oil content in rapeseed

A total of 11 QTL were detected by Windows QTL Cartographer 2.5 with composite interval mapping in BC_1 population, individually explaining 1.99-18.30% of the phenotypic variation (Table 6.1). Two QTL on A3, A5, A6 and C9 chromosome were identified. However, only one QTL was identified on A7, A10 and C5 chromosome. Six QTL on chromosomes A3, A6, A7 and C9 had negative additive effects of 1.43–4.49% for the variation of oil content, which deriving from the

10-Zi006 could decrease the oil content. In contrast, five QTL on chromosomes A5, A6, A10 and C5 with positive additive effects of 1.57-8.19% for the variation of oil content coming from high oil parent 10-4438. This result found two QTL simultaneously with opposite additive effects on A6 chromosome (Table 6.1, Figure 6.2). The distance of adjacent QTL on the chromosomes, such as A3, A5 and C9, was lower than 10cM, and the LOD score in each QTL ranged from 1.57 to 4.71 (Figure 6.2). The confidence intervals of each QTL spanned from 1.7cM to 9.6cM (Table 6.1).

Table 6.1 QTL for seed oil content in BC_1 population of the 10-4438 × 10- Zi006.

QTL	Chromosome	Position (cM)	LOD	Additive effect	R^2 (%)	CI[a]
qOC-a3-1	A3	93.91	4.71	-4.49	18.30	93.0-95.2
qOC-a3-2	A3	101.61	3.25	-4.34	14.42	101.0-102.7
qOC-a5-1	A5	83.81	2.48	3.99	9.08	82.3-85.8
qOC-a5-2	A5	93.61	2.32	8.19	13.79	92.6-94.7
qOC-a6-1	A6	133.71	2.61	-3.28	10.43	128.9-135.7
qOC-a6-2	A6	165.61	3.51	5.31	14.03	163.2-169
qOC-a7	A7	42.71	2.57	-1.71	11.85	40.3-44.1
qOC-a10	A10	93.21	2.53	3.38	11.59	92.1-94.5
qOC-c5	C5	135.71	2.61	1.57	1.99	135.7-145.3
qOC-c9-1	C9	17.61	1.57	-2.14	7.06	14.8-18.7
qOC-c9-2	C9	24.21	2.41	-1.43	11.36	20.9-28.6

[a] The 1-LOD confidence interval of QTL.

The previous studies disclosed the consensus QTL controlling the seed oil content in rapeseed were located on A1, A2, A3, A5, A6, A7, A8, A9, A10, C1, C2, C3,

C5, C6, C8 and C9 linkage groups (Jiang et al., 2014, Wang et al., 2013). However, A1 and A3 were detected to be the chromosomes that had the consistent locations of seed oil content QTL in different environment (Delourme et al., 2006, Zhao et al., 2012). Two QTL, *qOC-a3-1* and *qOC-a3-2*, were also identified in the BC_1 population of 10-Zi006 × 10-4438, indicating the QTL for oil content on the chromosomes A3 could be detected in various genetic background and less affected by environment. In addition, the QTL identified on chromosomes A2, A10 and C5 were considered only deriving from the population of high oil × low oil or medium oil content (Wang et al., 2013). Our results shown two QTL, *qOC-a10* and *qOC-c5*, were detected on chromosome A10 and C05, respectively, indicating high oil content parent 10-4438 may contain excellent alleles for oil content on this two chromosomes. Moreover, the first two QTL controlling the seed oil content on C09 linkage group were identified in DH population of zy036 ×51070 (Sun et al., 2012), Wang et al. (2013) also identified three oil QTL that located on C09 linkage group, in which one QTL was detected as a new QTL. The previous studies revealed the oil content QTL on C9 chromosome were always identified on the population that at least one parent with high oil content. Our result shown two QTL, *qOC-c9-1* and *qOC-c9-2*, on C9 chromosome coinciding with the previous studies.

The oil content in rapeseed, like other important agronomic traits in crops, is regulated by multiple genes. Although such complex genetic regulation makes it difficult to realize a substantial improvement in these traits through manipulation of a single gene, we try to map QTL to provide a genetic bases for improvement of those traits. However, previous studies revealed a relatively smaller contribution ranged from 1.2-19.0% for oil content with a single QTL (Chen et al., 2010, Delourme et al.,

2006, Ecke et al., 1995, Qiu et al., 2006, Zhao, J. et al., 2005). Even using the large segregating DH population with parents showing more than 10% difference in oil content, the single QTL was identified also just can explain 2.64–17.88% of the estimated phenotypic variation for oil content in rapeseed (Wang et al., 2013). Our results shown that the 1.99-18.30% of contribution for seed oil content variation was coincident with the previous studies, indicating the challenge for improvement of seed oil content in rapeseed especially due to the opposite additive effects of QTL on the same chromosome.

To compare the QTL regions on genetic linkage map with the physical map, the microsynteny was checked by aligning QTL regions with the reference genomes of *B. napus* L. (Figure. 6.3, Appendix Table 7). We found that 3.24cM and 3.39cM regions on A03 chromosome for seed oil content QTL (flanking markers, ‘SNP3080095’ and ‘SNP4331233’; ‘4167985’ and ‘SNP3096156’) were aligned to 3.05Mb and 11.55Mb genomic region on A03 of *B. napus* L. (from 13.12 to 16.17Mb and 4.66 to 16.20Mb), while a 6.06cM QTL region on linkage group A05 (flanking markers, ‘SNP3115424’ and ‘SNP4166042’) was aligned to a 0.26Mb region on chromosome A05 of *B. napus* L. (from 9.82 to 10.08 Mb). The QTL region for *qOC-a06-2* with 11.00cM on A06 linkage group (flanking markers, ‘SNP3076653’ and ‘3111491’) was aligned to 2.76Mb region on chromosome A06 of *B. napus* L. (from 16.88 to 19.64Mb). On A10 linkage group, *qOC-a10* with 2.85cM (flanking markers, ‘SNP5239050’ and ‘ 4331484’) was aligned to 0.33Mb region on chromosome A10 of *B. napus* L. (from 11.81 to 12.14Mb). two QTL regions on C09 linkage group with 5.56cM and 8.86cM (flanking markers, ‘3154062’ and ‘3099341’;

‘3085977’ and ‘SNP3177178’) were aligned to 11.44Mb 1.72Mb regions on C09 of *B. napus* L. (from 21.06 to 32.50Mb and 19.34 to 21.06Mb). However, the flanking marker or linkage markers for *qOC-a6-1*, *qOC-a10* and *qOC-c5* could not aligned well to the *B. napus* L. genome.

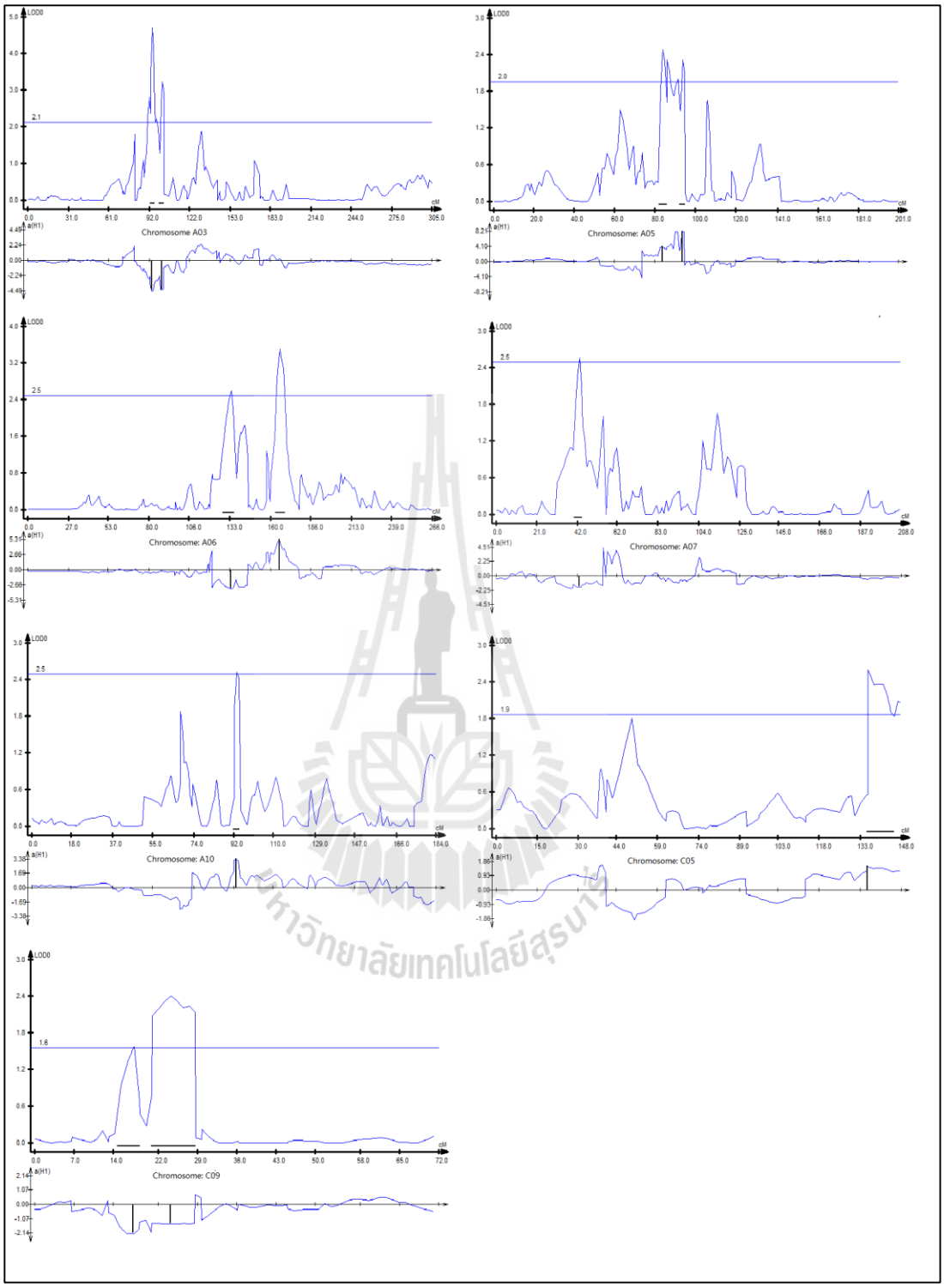

Figure 6.2 Oil content QTL in BC_1 population of 10-4438 × 10- Zi006 identified through the whole genome analysis in *B. napus* L..

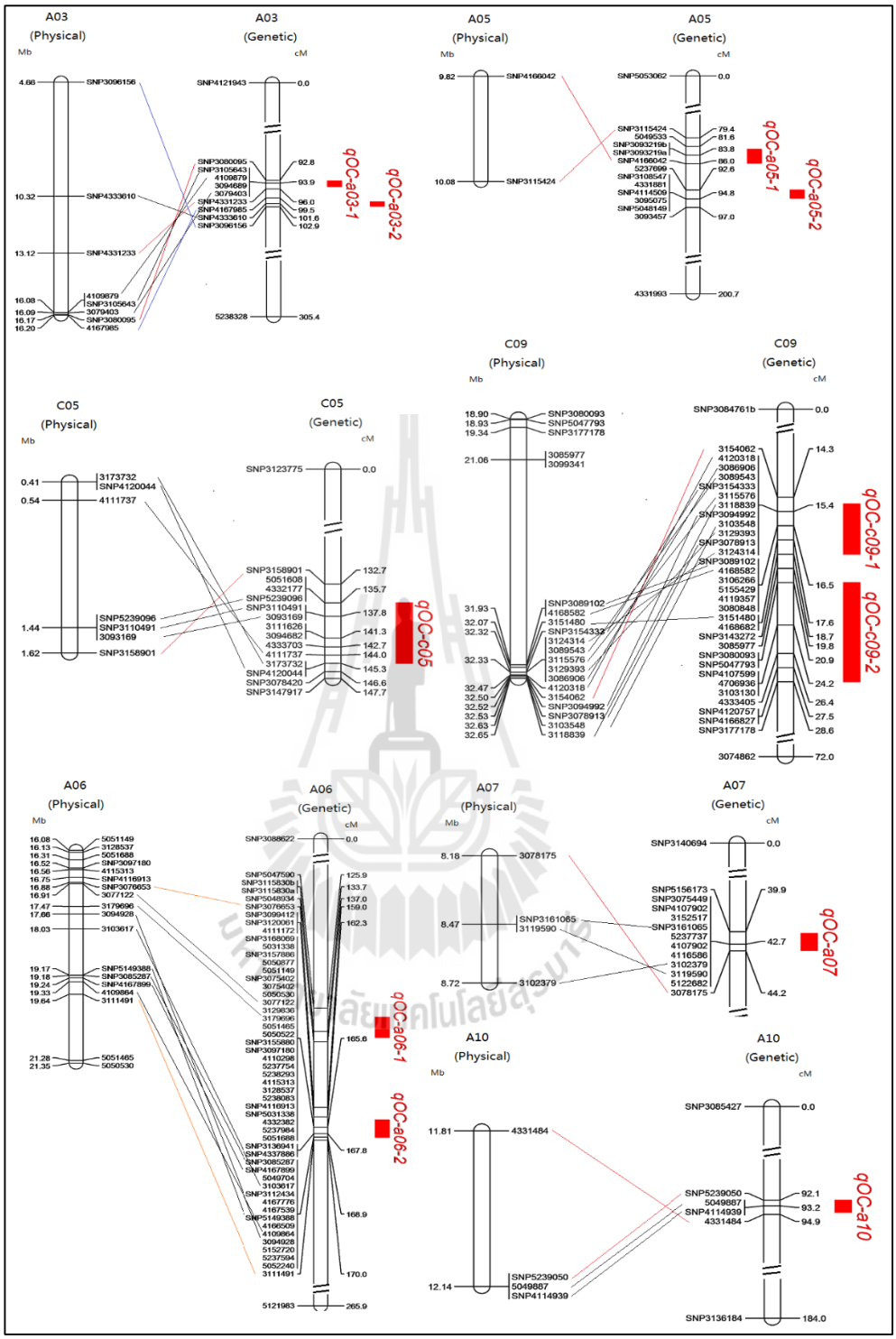

Figure 6.3 Locations of putative QTL for oil content on the high density genetic linkage map of 10-4438 × 10- Zi006 with microsynteny analyses of QTL regions by aligning to reference genomes of *B. napus* L

6.4.3 Mapping QTL controlling the leaf anthocyanin content

A total of 14 QTL were detected to control the leaf anthocyanin content by Windows QTL Cartographer 2.5 with composite interval mapping in BC_1 population of 10-Zi006 × 10-4438, and individually explaining 9.17-19.13% of the phenotypic variation (Table 6.2).

Table 6.2 QTL for leaf anthocyanin content in the 10-4438 × 10- Zi006 BC_1 population.

QTL	Chromosome	Position(cM)	LOD	Additive effect	R^2(%)	CI[a]
qAC-a03-1	A3	59.81	4.66	0.25	19.13	58.4-60.9
qAC-a03-2	A3	74.21	3.08	-0.22	11.47	73.2-76.3
qAC-a03-3	A3	179.51	3.68	-0.24	13.77	177.6-181.4
qAC-a03-4	A3	188.31	2.37	-0.20	9.17	186.1-190.4
qAC-a04	A4	66.21	2.64	0.18	12.21	65.2-67.4
qAC-a05-1	A5	152.21	4.11	0.30	17.86	151.2-153.4
qAC-a05-2	A5	163.11	2.87	-0.22	12.19	162.7-165.5
qAC-a05-3	A5	168.81	2.26	-0.14	9.35	167.7-171.7
qAC-a06-1	A6	209.11	3.35	0.15	14.32	208.5-211.7
qAC-a06-2	A6	221.11	2.67	-0.21	12.76	220.9-222.3
qAC-c02	C2	66.41	2.05	-0.19	9.58	65.3-70.2
qAC-c04-1	C4	54.91	3.30	-0.16	14.11	54.7-58.3
qAC-c04-2	C4	64.71	3.03	-0.16	13.05	61.4-64.8
qAC-c04-3	C4	119.51	3.12	0.20	12.68	118.6-122.1

[a] The 1-LOD confidence interval of QTL.

Four QTL were identified on A3 chromosome, while three QTL were detected on A5 and C4 chromosomes. Two QTL on A6 and one QTL on A4 and C2 chromosomes were also identified. Five QTL on chromosomes A3, A4, A5, A6 and C4 had positive additive effects of 0.15–0.30 (A_{530}-0.25×A_{657}) /g for the variation of leaf anthocyanin content, which deriving from the 10-Zi006 and could increase the anthocyanin content in rapeseed leaf. In contrast, nine QTL on chromosomes A3, A5, A6 and A4 with negative additive effects of 0.14-0.24 (A_{530}-0.25×A_{657}) /g derived from lower anthocyanin parent 10-4438. The LOD score in each QTL ranged from 2.26 to 4.66 and the confidence intervals of each QTL spanned from 1.4 to 4.9 cM (Table 6.2, Figure 6.4).

In *B. campestris* L., a number of purple genes had been mapped. One purple gene in Chinese cabbage had been mapped on linkage group A09 and located between two RAPD marker loci with interval of 4.92cM (Zhang et al., 2008). Red anthocyanin pigment locus (*Anp*) in red turnip was identified on linkage group A07 with SSR marker (Hayashi et al., 2010). Moreover, QTL for seedling anthocyanin (*RLAn12-157*) was also identified on chromosome A07 at 12.027 Mbp interval. Candidate locus AT3G59030 has a significant hit within this region at 12.82 Mbp on *B. rapa* genome with an annotation of *Transparent Testa12 (TT12)* (Rahman et al., 2014). Another purple leaf gene locus, *BrPur*, was mapped on linkage group A03 of *B. rapa* and was defined the interval with genetic interval of 0.6 cM and a genomic region of 54.87 kb (Wang, Weihong et al., 2014). Our studies revealed four QTL, *qAC-a03-1*, *qAC-a03-2*, *qAC-a03-3* and *qAC-a03-4* on chromosome A03. However, none of the QTL had been identified on A07 and A09 chromosomes, but ten QTL were detected on A03, A04, A05, A06, C02 and C04 chromosomes. Despite only one dominance

gene was identified to control the red turnip, the pigmentation were found to vary within the same genotype indicating the color intensity could be controlled by other loci in addition to the *Anp* locus (Hayashi et al., 2010). Our results confirmed the intensity of pigmentation controlled by polygene loci by QTL mapping with DArT-seq markers.

The DArT-seq technique as a powerful tool provided large number of SNP and *in silico* DArT polymorphic markers to construct high-density genetic linkage map for QTL mapping. It was convenient to align the genetic map with the physical map and match the QTL regions to the physical map. The majority of QTL controlling the leaf anthocyanin content were well matched to the physical map (Figure 6.5, Appendix Table 8). Three QTL region on A3 linkage groups were matched to A3 chromosome and physically defined to intervals of 13.11, 0.23 and 14.54Mbp, respectively. However, one QTL on A3 linkage groups was matched to C3 chromosome and physically defined to intervals of 0.21Mbp. Three QTL regions on A5 linkage group were matched to A5 chromosome and physically spanned intervals of 0.14, 0.25 and 0.50Mbp, respectively. However, none of marker was aligned on the physical map region in which the *qAC-a05-1* was mapped from 20.84 to 20.98Mbp between markers ‘SNP3215026’ and ‘4337489’. One QTL on each A04, C02 and C04 linkage group was matched to A04, C02 and C04 chromosome and spanned physically intervals of 2.28, 8.46 and 0.02Mbp. Another two QTL regions on A06 genetic linkage group were matched physically to C07 chromosome and defined to intervals of 0.52 and 0.21 Mbp, respectively.

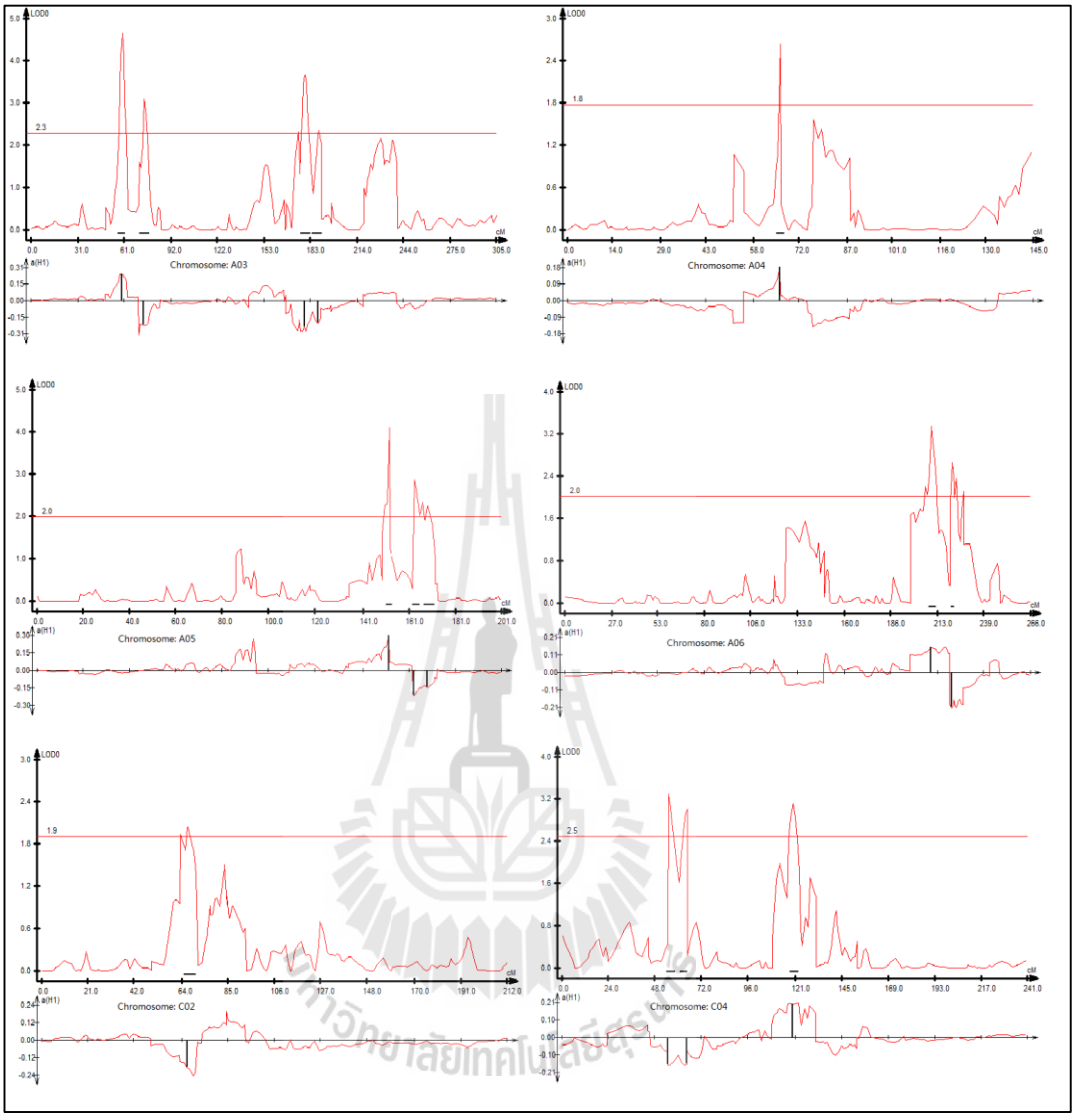

Figure 6.4 Anthocyanin content QTL in BC_1 population of 10-4438 × 10- Zi006 identified through the whole genome analysis in *B. napus* L

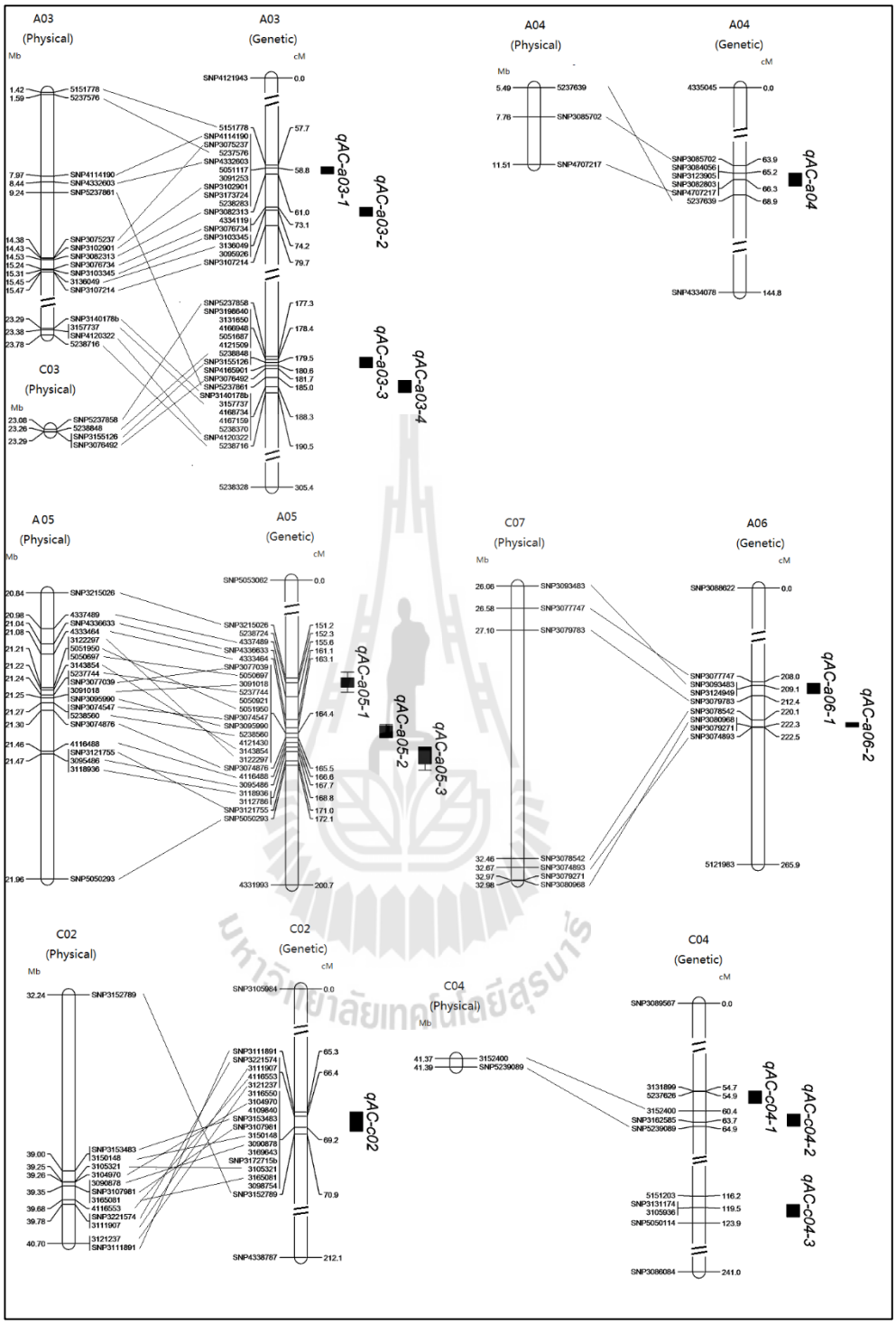

Figure 6.5 Locations of putative QTL for anthocyanin content on the high density genetic linkage map of 10-4438 × 10- Zi006 with microsynteny analyses of QTL regions by aligning to reference genomes of *B. napus* L.

6.4.4 Mapping QTL controlling the leaf chlorophyll content

A total 8 QTL associated with leaf chlorophyll content were identified on seven linkage groups, contributing individually 4.45% to 13.07% of phenotypic variation (Table 6.3, Figure 6.6 and 6.7). Among those QTL, two QTL were located on A3 linkage group at 290.61cM and 300.91cM, named *qCC-a03-1* and *qCC-a03-2*. Single QTL was identified on each A1, A6, A7, C1, C3 and C4 linkage group at 178.71cM, 165.61cM, 135.51cM, 30.01cM, 190.81cM and 41.41cM, and were named as *qCC-a01*, *qCC-a06*, *qCC-a07*, *qCC-c01*, *qCC-c03* and *qCC-c04*, respectively.

Three QTL, *qCC-a03-1*, *qCC-a06* and *qCC-c03* with positive additive effect could increase the leaf chlorophyll content deriving from 10-Zi006. Another five QTL with negative additive effect could decrease the leaf chlorophyll deriving from lower chlorophyll content parent 10-4438.

The confidence interval of the QTL ranged from 1.4 to 5.9 cM (Table 6.3). The *qCC-a01* with 3.1cM of interval located between SNP3085963 and 3161450 and was tightly linked with one marker locus (two markers). The *qCC-a03-1* (confidence interval of 2.1cM) with one locus (30 markers) was anchored between SNP4121170 and 5238312, while the *qCC-a03-2* with 2.1cM interval located between 5238833 and SNP3148583 and associated with one locus (7 markers). The *qCC-a06* (flanking markers: ‘SNP3076653’ and ‘SNP4337886’, 3.6cM of confidence interval) was tightly linked with one locus (26 markers). The *qCC-a07* (flanking marker: ‘3178737’ and ‘SNP3080129’), *qCC-c01* (flanking marker: ‘SNP3111957’ and ‘3080193’), *qCC-c03* (flanking marker: ‘SNP3122036’ and ‘SNP5053562’), *qCC-c04* (flanking marker: ‘SNP5048038’ and ‘3108980’) with confidence of intervals 5.9, 1.4, 1.7 and 3.7 cM were linked with three marker loci (13 markers), one locus (9 markers), two

loci (15 markers) and two loci (3 markers), respectively.

Table 6.3 QTL for leaf chlorophyll content in the 10-4438 × 10- Zi006 BC_1 population.

QTL	Chromosome	Position(cM)	LOD	Additive effect	R^2(%)	CI[a]
qCC-a01	A1	178.71	2.93	-9.20	11.87	176.9-180.0
qCC-a03-1	A3	290.61	2.86	7.89	11.35	288.6-290.7
qCC-a03-2	A3	300.91	2.51	-7.38	13.07	299.8-301.9
qCC-a06	A6	165.61	2.58	7.85	10.67	163.8-167.4
qCC-a07	A7	135.51	2.81	-4.99	12.44	132.8-138.7
qCC-c01	C1	30.01	1.89	-5.50	8.56	29.5-30.9
qCC-c03	C3	190.81	2.46	8.86	4.45	190.7-192.4
qCC-c04	C4	41.41	2.63	-5.32	11.15	40.2-43.9

[a] The 1-LOD confidence interval of QTL.

The chlorophyll is one of the major pigments in chloroplast for photosynthesis, and the chlorophyll content has been shown to have a positive relationship with photosynthetic rate (Avenson et al., 2005, Wang et al., 2003). Thus, the identification of QTL or genes involved in chlorophyll biosynthesis as well as thylakoid membrane and chloroplast biogenesis could be applied to improve energy conversion efficiencies (Melis, 2009, Ort et al., 2011). The QTL or genes seemed to be anchored on linkage group A01 and A06 in *Brassica* species. A locus, *BnChd1-1*, involved in chlorophyll biosynthesis in *B. napus* was mapped to a region of A01 using a BC_3F_1 population, and an candidate gene was identified for *BnChd1-1* (Zhao et al., 2014). Studies on seeding chlorophyll for *B. rapa* shown that one QTL were detected

on each A01 and A06 linkage groups, respectively (El-Soda et al., 2014). In Chinese cabbage, QTL controlling the chlorophyll b was identified on A01 and A06 linkage groups both with negative additive effect (Ge et al., 2012). We also identified a QTL on A01 linkage group at 178.71cM with LOD score of 2.93, which could explain 11.87% of chlorophyll phenotypic variation. This QTL region was matched to the physical map with interval of 1.75 Mbp (flanking marker: 'SNP3085963' and '3161450', from 14.23 to 15.98 Mbp), and was tightly linked with the marker SNP3151854 at 14.61Mbp. Another QTL was detected on A06 linkage group with positive additive effect, and the QTL region was tightly linked with ten markers physically ranged from 16.08 Mbp to 21.35 Mbp (Figure 6.7, Appendix Table 9).

Our results were similar to the previous studies showing the same QTL locations for chlorophyll content. Such as the QTL controlling chlorophyll a and b had been identified on linkage group A03 and A07 (Ge et al., 2012). We also found two QTL associated with chlorophyll content on linkage group A03, and one QTL, the *qCC-a03-2*, was anchored physically in interval of 2.96 Mbp on A03 chromosome (flanking marker: '5238833' and 'SNP3148583', from 2.88 to 5.84Mbp). However, the *qCC-a03-2* was matched not only to chromosome A03 but also to chromosome C07 (Figure 6.7). The QTL that located on A07 linkage group was matched on physical map with an interval of 0.80 Mbp, tightly linkage with five markers (SNP3081064 17.71Mbp, SNP3092737 17.71Mbp, 5051741 17.74Mbp, SNP3100378 17.88Mbp, 3155716 17.92Mbp) (Appendix Table 9).

In addition to A genome, three QTL were identified in C genome. The QTL regions of *qCC-c01* and *qCC-c03* were matched physically on intervals of 0.48 and 1.44 Mbp from 10.82 to 11.30 Mbp and 49.14 to 50.58 Mbp, respectively. However

the QTL region of *qCC-c04* could not well match to the physic map.

This result indicated that QTL for seed oil and leaf chlorophyll content with partially overlapping confidence interval were detected on A06 linkage group. Ten markers (5051149, 16.08Mbp; 5050530, 21.35Mbp; 3077122, 16.91Mbp; 3179696, 17.47Mbp; 5051465, 21.28Mbp; SNP3097180, 16.52Mbp; 4115313, 16.56 Mbp; 3128537, 16.13Mbp; SNP4116913, 16.75Mbp; 5051688, 16.31Mbp) were tightly and simultaneously linked with the *qOC-a06-2* and *qCC-a06*. In particular, both of the two QTL had positive additive effect indicating this locus could increase the seed oil content and leaf chlorophyll content simultaneously in rapeseed. The similar QTL with overlapping confidence interval was detected for flowering time and *Sclerotinia sclerotiorum* resistance in the field on A02, A03 and C02 linkage group (Wei et al., 2014).

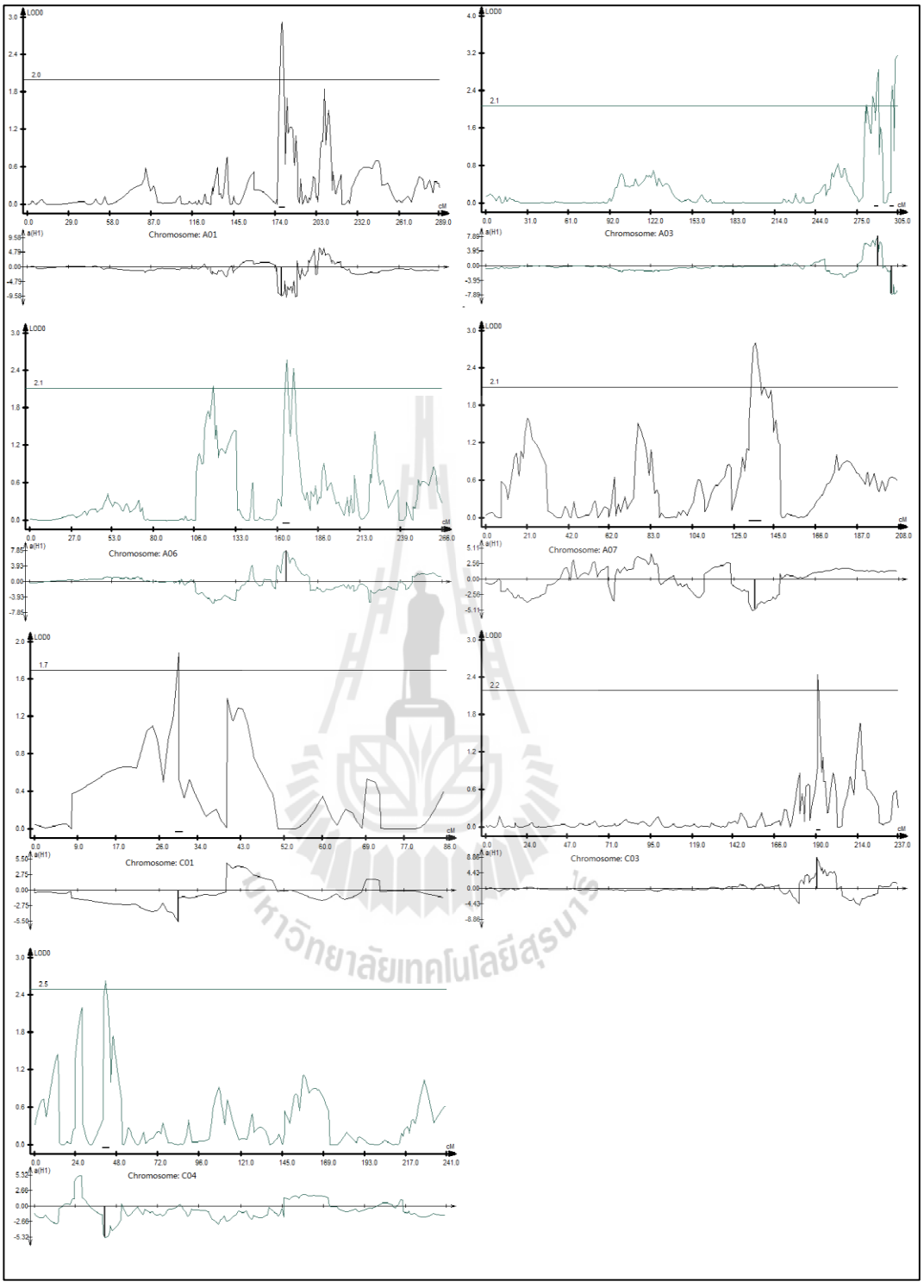

Figure 6.6 Locations of putative QTL for chlorophyll content on the high density genetic linkage map of 10-4438 × 10- Zi006 with microsynteny analyses of QTL regions by aligning to reference genomes of *B. napus* L.

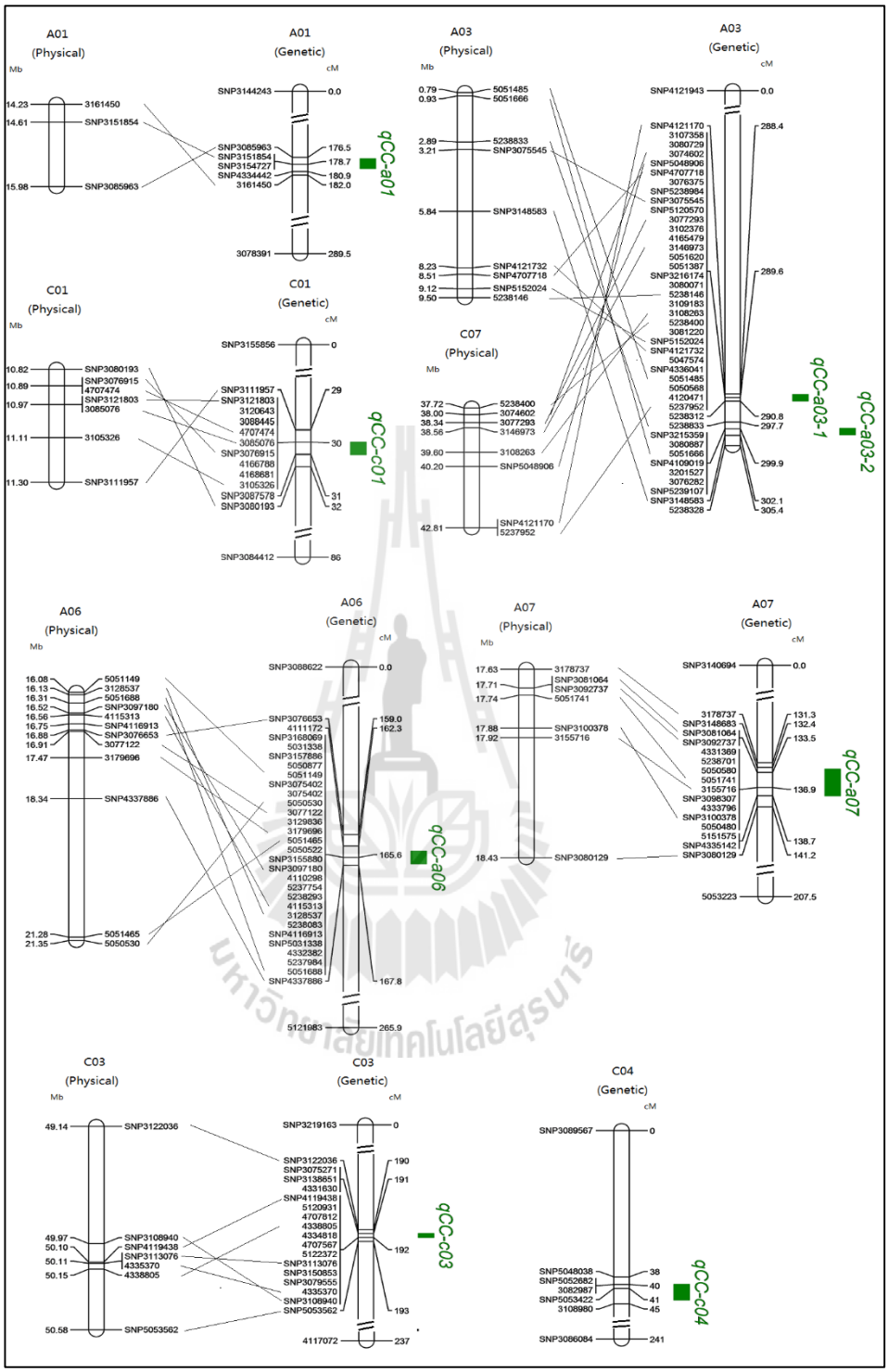

Figure 6.7 Locations of putative QTL for anthocyanin content on the high density genetic linkage map of 10-4438 × 10- Zi006 with microsynteny analyses of QTL regions by aligning to reference genomes of B. napus L.

6.5 Conclusion

In this study, we have identified total number of 11, 14, and 8 QTL that controlled the seed oil, leaf anthocyanin and chlorophyll contents in rapeseed, respectively. Those QTL could explain the phenotypic variation for the study traits ranged from 1.99 to 18.30%, 9.17 to 19.13% and 4.45 to 13.07%, respectively. It was a power tool with the high-density genetic linkage map that could allowed adjacent QTL to be mapped on the same chromosome with distance less than 10 cM. However, those adjacent QTL tended to be with opposite additive effect. The most QTL location were consistent with the previous studies, such as chromosome A03 detected in our BC_1 population were considered to be the consistent locations for seed oil content in different environment. However, due to the small population size, maybe the limitation on number of QTL had been detected to control the study traits, such as the A09 and A10 were identified with chlorophyll content QTL in *B. rapa* (El-Soda et al., 2014, Ge et al., 2012), but we were failed identified the QTL on those two linkage groups. In addition, the DArT-seq technique provided an alignment between the marker sequence and the *B. napus* genome. Those information could allow us to match the QTL regions to the physical map region, most of the QTL associated with the study traits were well anchored physically on the intervals of 0.02 to 14.54Mbp. Moreover, the QTL were tightly linked with a few marker loci, in which some markers were aligned to the physical map with particular position. The results were useful on detecting the candidate genes and map-based cloning and marker assistant selection for the traits.

6.6 Reference

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CHAPTER VII

CONCLUSION

The genetic analysis for seed oil, leaf anthocyanin and chlorophyll contents in rapeseed were studied and the results as follows:

1. The generation mean analysis revealed that seed oil content in rapeseed controlled by non-allelic interaction. Six parameters analysis indicated that seed oil content controlled by m + [d] + [i] + [l] and the additive effect contributed highly significant to the inheritance of oil content, followed by dominance × dominance epistatic effect. The major gene plus polygene analysis showed that a mixture of two additive-dominant-epistasis major genes plus additive-dominant polygene controlled the oil content with a magnitude of positive additive effect and dominance effect of polygenes. The genetic variance in BC_1 and BC_2 mainly resulted from the major genes. However, the genetic variance in F_2 resulted from both major gene and polygene. The lower heritability for polygene and major gene were detected in BC_1, BC_2 and F_2 generation indicating additive and non-additive effect were important for oil content in rapeseed.

2. Analysis of variance for generation mean showed that leaf anthocyanin content were significant different between years, generations and year × generations interaction. Six parameters analysis revealed that the leaf anthocyanin content mainly controlled by additive effect, additive × additive, additive × dominance and dominance × dominance interaction effect. However, the greatest magnitude of gene

effects for anthocyanin content was dominance × dominance, followed by additive and additive × additive effect in 2013 while dominance × dominance effect was the greatest followed by additive × additive and additive effect in 2014. In addition, the epistatic effects were higher than the main effects and the total fixable gene effect was higher than total non-fixable gene effect in the genetic control of anthocyanin content. The analysis of twelve parameters model showed that the additive effect, additive × additive effect and dominance × dominance effect were highly significant, and environmental effect and environmental × additive effect were significant in anthocyanin inheritance and m+ [d] + [h] + [i] + [l] + el + gdl + gil was the perfect model for genetic control of anthocyanin. High broad sense heritability and moderate narrow sense heritability were obtained for anthocyanin content indicating this character was controlled mainly by genetic effect, and both additive and non-additive gene effects were important.

3. The analysis of variance for generation mean showed the leaf chlorophyll content were significant different between years and generations, but was not significant different between year × generations interaction. Six parameters analysis revealed the leaf chlorophyll content mainly controlled by additive effect, dominance effect, additive × additive effect, additive × dominance and dominance × dominance effect. The greatest magnitude of gene action for chlorophyll content was dominance × dominance effect, and followed by additive × additive and dominance effect in 2013 while additive ×additive was the greatest effect followed by dominance × dominance and dominance effect in 2014. In addition, the epistatic effects were higher than the main effects and the total fixable gene effects was lower than total non-fixable gene effects in the genetic control of chlorophyll content. The analysis of twelve

parameters model showed the additive effect, dominance effect, additive × additive effect, additive × dominance effect, dominance × dominance effect and environmental effect were highly significant and the fitness genetic model for chlorophyll content was m+ [d] + [h] + [i] + [j] + [l] + el. Moderate broad sense heritability and lower narrow sense heritability were detected for chlorophyll content indicating this trait was affected by genetic and non-genetic effect, and both the additive affects and non-additive affects as well as environmental effects were important.

4. The correlation analysis between leaf anthocyanin and chlorophyll contents indicated that the correlation differed between generations. The negative correlation existed in the populations with higher anthocyanin content (P_1, F_2 and BC_1) and positive correlations were existed in the populations with lower anthocyanin content (P_2, F_1 and BC_2).

5. In this study, DArT-seq was used as a novel technique for genotyping through whole genome profiling. The total of 9372 polymorphic markers including 5968 *in silico* DArT markers and 3404 SNP markers were detected by DArT-seq, which was the prerequisite for high-density map construction. In addition, the high-quality SNP markers were firstly selected for genetic linkage framework map construction and the use of the 'fixed order' function in JionMap v4.0 was a way to ensure the right markers order in high-density map, which was subsequently constructed by attaching the remaining polymorphic markers into the framework map. Moreover, the program SMOOTH combined with JionMap v4.0 to remove the genotyping error was an efficient way to decrease the ordering ambiguities between markers, and the Online MST_{MAP} was a novel online program for clustering and ordering thousands of markers efficiently. The high-density genetic linkage map was

finally constructed containing 1614 discrete loci with 9212 markers, and covering 3616.38 cM with average genetic distance 2.24 cM between loci. This genetic linkage map was useful for genetic studies, such as QTLs identification for interesting traits in rapeseed. However, one of the parent 10-Zi006 had been developed by distant hybridization, which maybe result in the chromosomal rearrangements, gene conversation and/or transposition, etc. We can reasonably infer that this maybe the reason of resulting in segregation distortion, especially all the distorted markers on A2 linkage group.

6. Continuously distribution for seed oil, leaf anthocyanin and chlorophyll contents indicated that these traits were quantitative inheritance. The identification of QTL showed a total of 11 QTL controlled seed oil content on linkage groups A03, A05, A06, A07, A10, C05 and C09. The confidence intervals of each QTL spanned from 1.7 to 9.6 cM, individually explaining 1.99-18.30% of the phenotypic variation. Of which, the *qOC-a3-1* and *qOC-a3-2* would be less affected by environment due to the previous studies showed that QTL for oil content on A1 and A3 linkage groups was consensus QTL in different environments. In addition, two QTL were identified on A10 and C05 linkage groups which were considered to be derived from the parent with high oil content. Thus, we could ensure that the high oil content parent 10-4438 contained novel alleles controlling high oil content in rapeseed. Identification of QTL for anthocyanin content showed 14 QTL located on A03, A04, A05, A06, C02 and C04 linkage groups individually explaining 9.17-19.13% of the phenotypic variation. The LOD score in each QTL ranged from 2.26 to 4.66 and the confidence intervals of each QTL spanned from 1.4 to 4.9 cM. For leaf chlorophyll content, a total of 8 QTL were identified on A01, A03, A06, A07, C01, C03 and C04 linkage groups. The

confidence interval of those QTL ranged from 1.4 to 5.9 cM and individually explained 4.45% to 13.07% of phenotypic variation. We also identified a partially overlapping confidence interval on A06 linkage group for QTL associated with seed oil and leaf chlorophyll content. Ten markers were tightly and simultaneously linked with the *qOC-a06-2* and *qCC-a06*. In particular, both QTL had positive additive effect indicating this locus could increase the seed oil content and leaf chlorophyll content simultaneously in rapeseed.

7. DArT-seq technique provided an alignment between the marker sequence and the *Brassica napus* genome. Those information could allow us to match the QTL regions to the physical map region. Most of the QTL were well physically anchored on the related chromosomes with intervals of 0.02 to 14.54 Mbp. Moreover, the QTL were tightly linked with few marker loci, in which some markers were aligned to the physical map with particular position. The results were useful on detecting the candidate genes and map-based cloning and marker assistant selection for the traits

APPENDICES

APPENDIX A

THE PROCEDURE AND REAGENTS FOR DNA ISOLATION

Table 1 The procedure of modified CTAB DNA isolation.

Step	Content
1	Grind 0.2g of young leaf tissue in centrifuge tube (2ml) under liquid nitrogen to fine powder using a Geno/Grinder™ 2000 (SPEX Sample Preparation, USA).
2	Suspend powder in 0.8 ml pre-warmed (65 °C) CTAB + 2% β-mercaptoethanol buffer solution.
3	Incubate at 65ºC for 45min, invert tubes in every 15 minutes or incubate with gentle shaking.
4	Cool down for 5 min and transfer about 0.6ml of the supernatant to fresh tube (2ml).
5	Add 0.6 ml of chloroform : isoamyl alcohol (24 : 1) mixture, and mix well for 20 min with gentle shaking.
6	Spin 10 min, 1200rpm, RT
7	Transfer water phase to fresh tube, add 0.4 ml of ice cold isopropanol and store at 4℃ (or -20 ℃) for 30 min, nucleic acids should become visible.
8	Spin 5 min, 1000rpm, RT
9	Discard supernatant, wash pellet with 1 ml 75 % EtOH for 10 min.
10	Spin 3 min, 800rpm, RT
11	Discard EtOH, wash pellet with 1 ml 100 % EtOH for 10 min.
12	Discard EtOH, dry pellet and dissolve in 200µl 1 × TE (Table 7.4) store at 4℃ for 24-48 h.
13	Check DNA quality and quantity by NanoDrop 1000 (NanoDrop Technologies, Houston, TX, USA).and on 1% TAE buffered agarose gel.

Table 2 The components and amount in 500ml of CTAB buffer (2% w/v)

Components	Concentration (w/v, M)	Amount (g, ml)
CTAB	2%	10 (AR)
NaCl	1.4	40.948 (AR)
EDTA	0.02	20 (0.5M, PH=8.0)
Tris-HCl	0.1	50 (1M, PH=8.0)
ddH_2O	Volume to 500 ml	

Table 3 The components and amount in 500ml of Tris-HCl (1M, PH=8.0)

Components	Amount (g, ml)
Tris	60.55 (AR)
Concentrated HCl	21
ddH_2O	Volume to 500 ml and adjust PH equal to 8.0

Table 4 The components and amount in 500ml of EDTA (0.5M, PH=8.0)

Components	Amount (g)
EDTA	93.06 (AR)
NaOH	10
ddH_2O	Volume to 500 ml and adjust PH equal to 8.0

Table 5 The components and amount in 500ml of TE (1×, PH=8.0)

Components	**Amount (ml)**
Tris-HCl (1M, PH=8.0)	5
EDTA(1M, PH=8.0)	1
ddH_2O	Volume to 500 ml and adjust PH equal to 8.0

APPENDIX B

THE DART MARKER AND GENETIC LINKAGE MAP

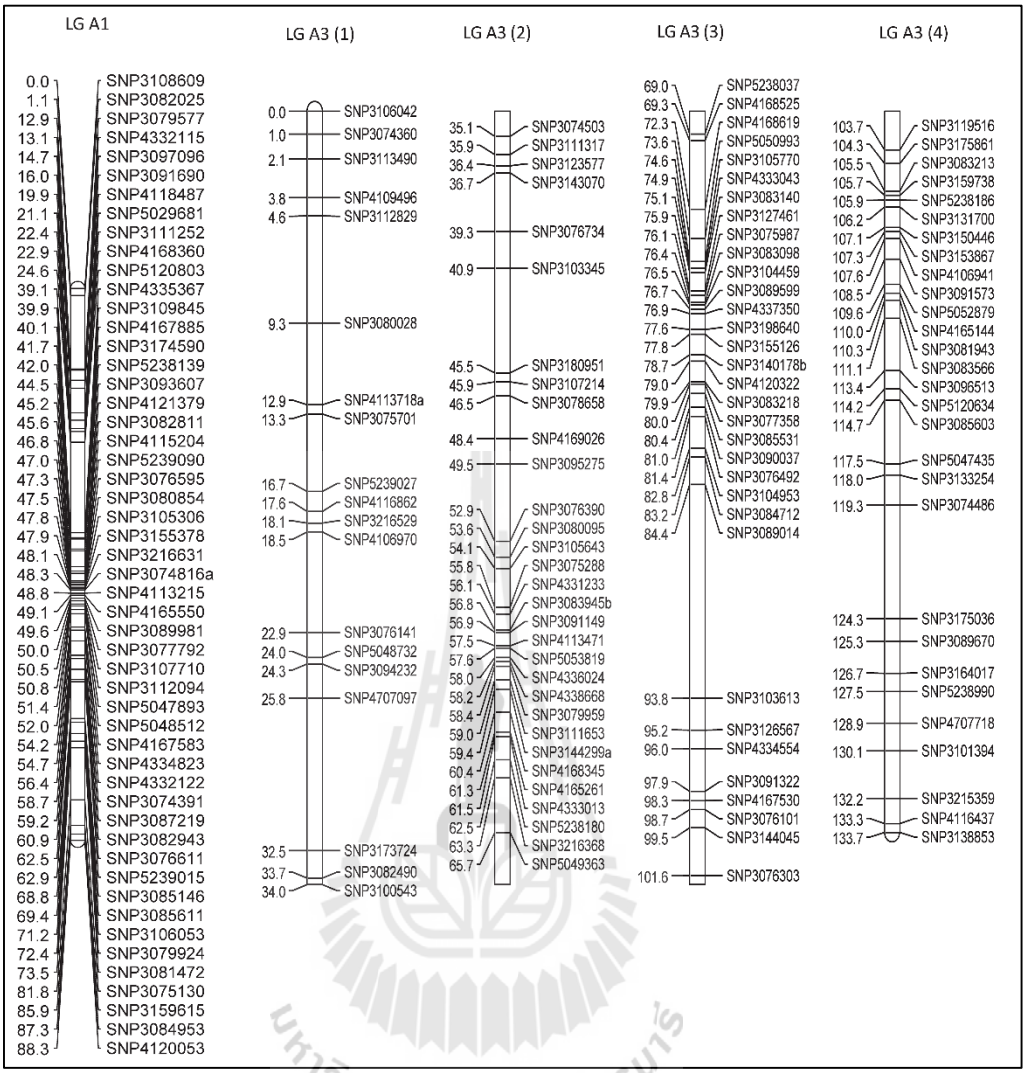

Figure 1 The genetic linkage framework map (Map 1) of BC_1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

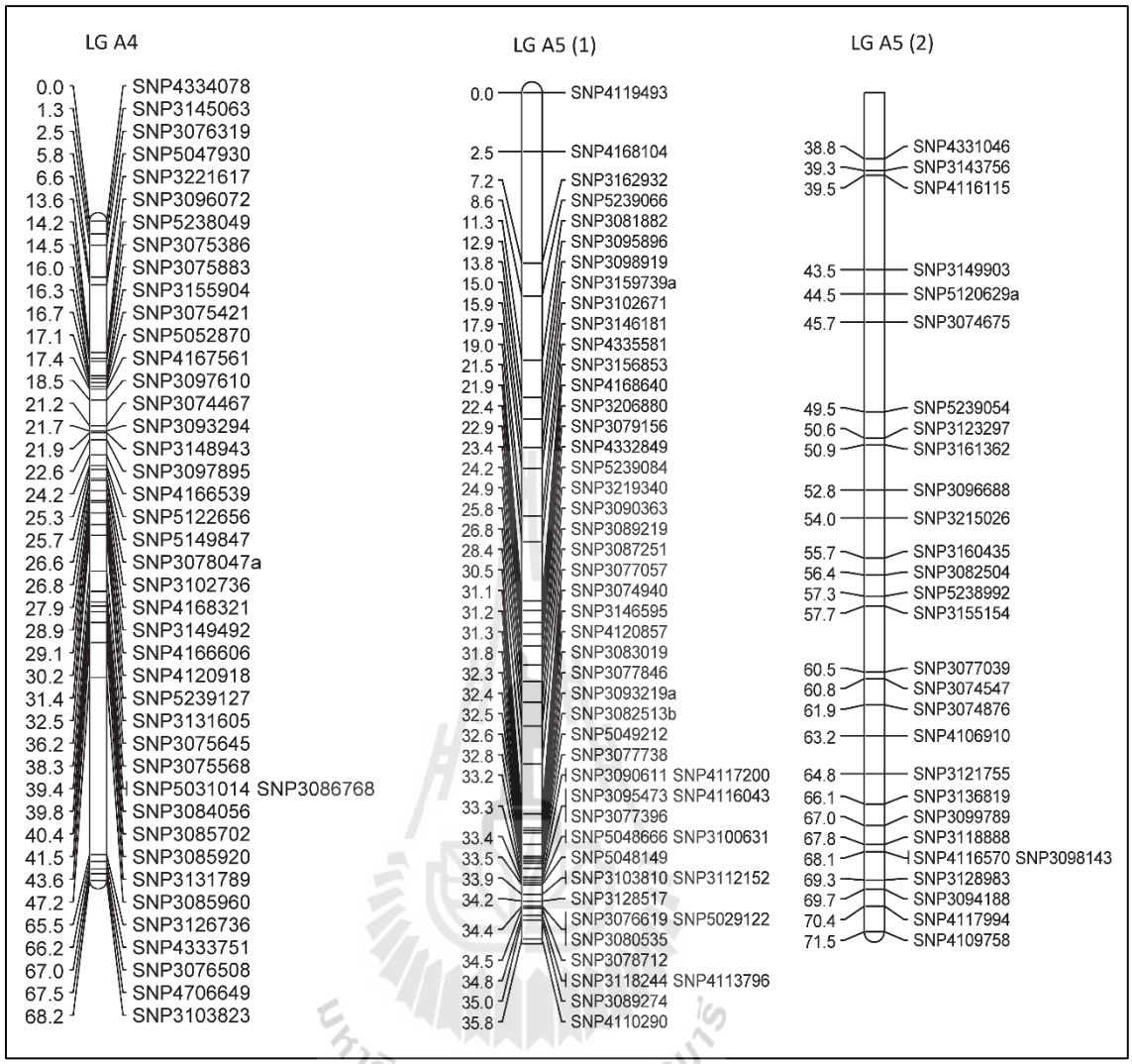

Figure 1 (Continued) The genetic linkage framework map (Map 1) of BC1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

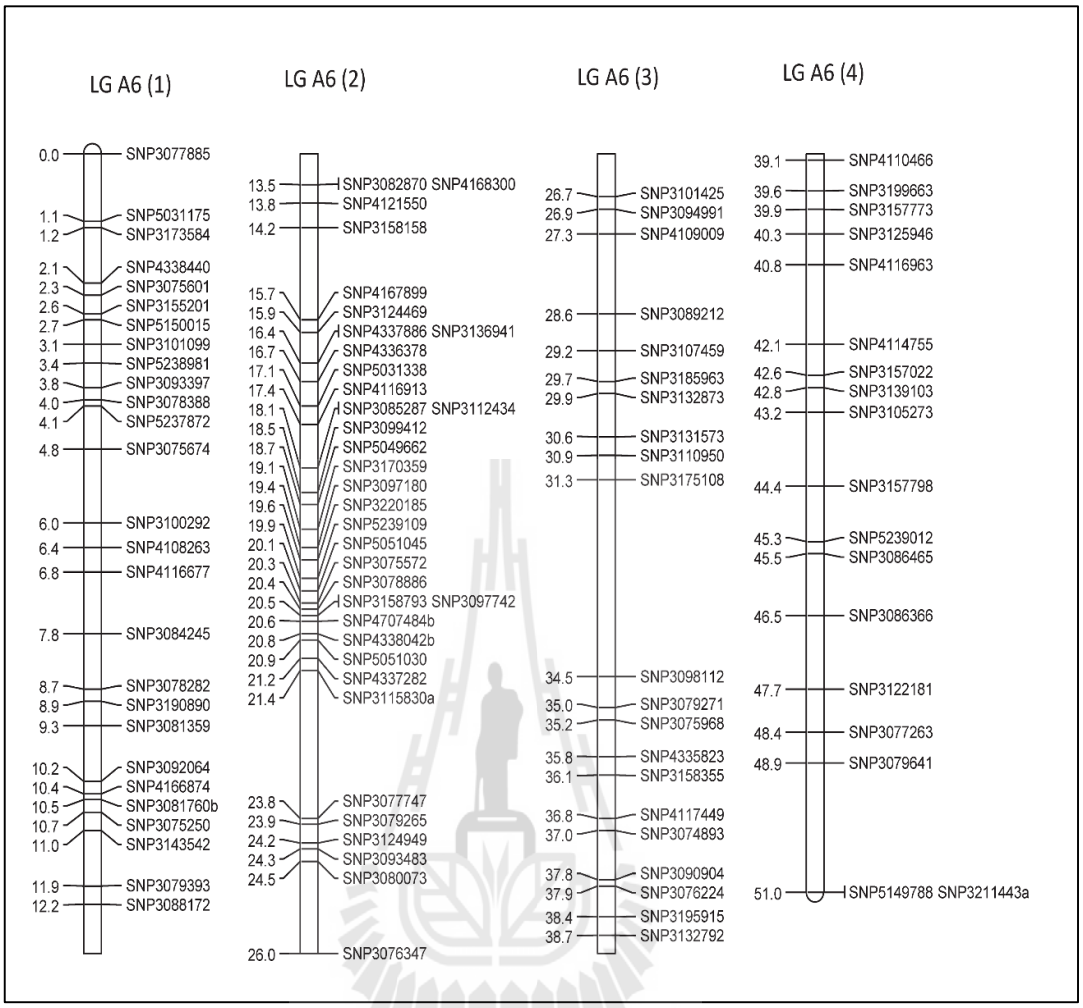

Figure 1 (Continued) The genetic linkage framework map (Map 1) of BC_1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

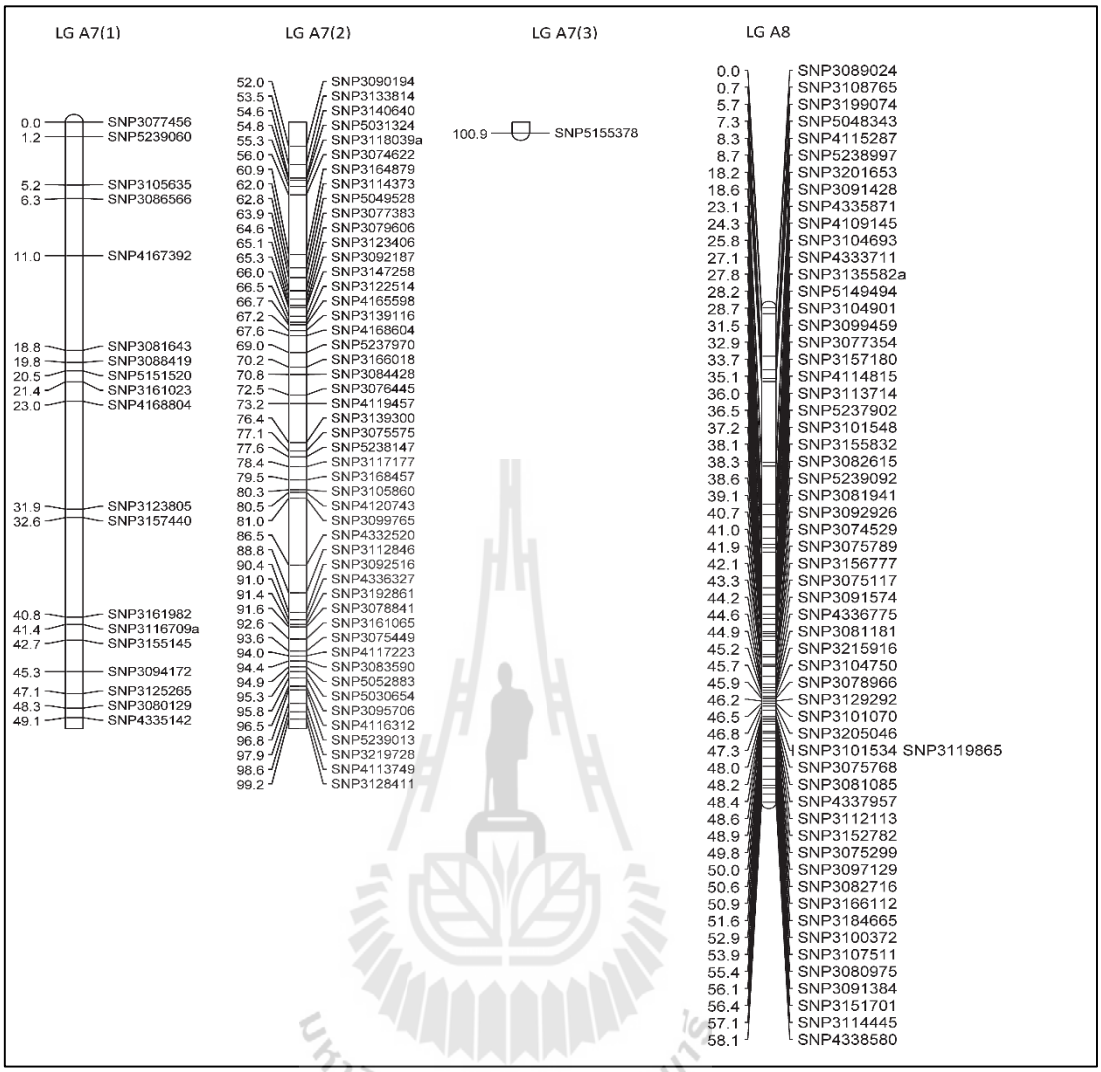

Figure 1 (Continued) The genetic linkage framework map (Map 1) of BC_1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

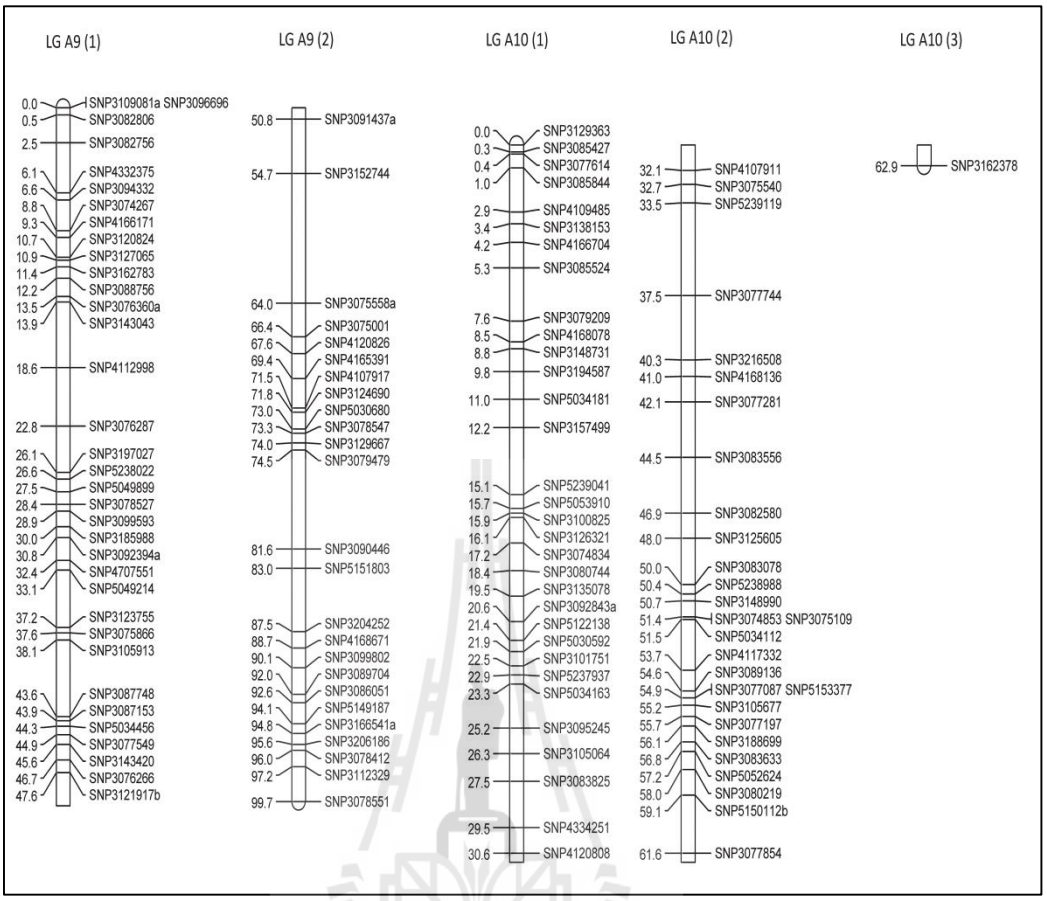

Figure 1 (Continued) The genetic linkage framework map (Map 1) of BC_1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

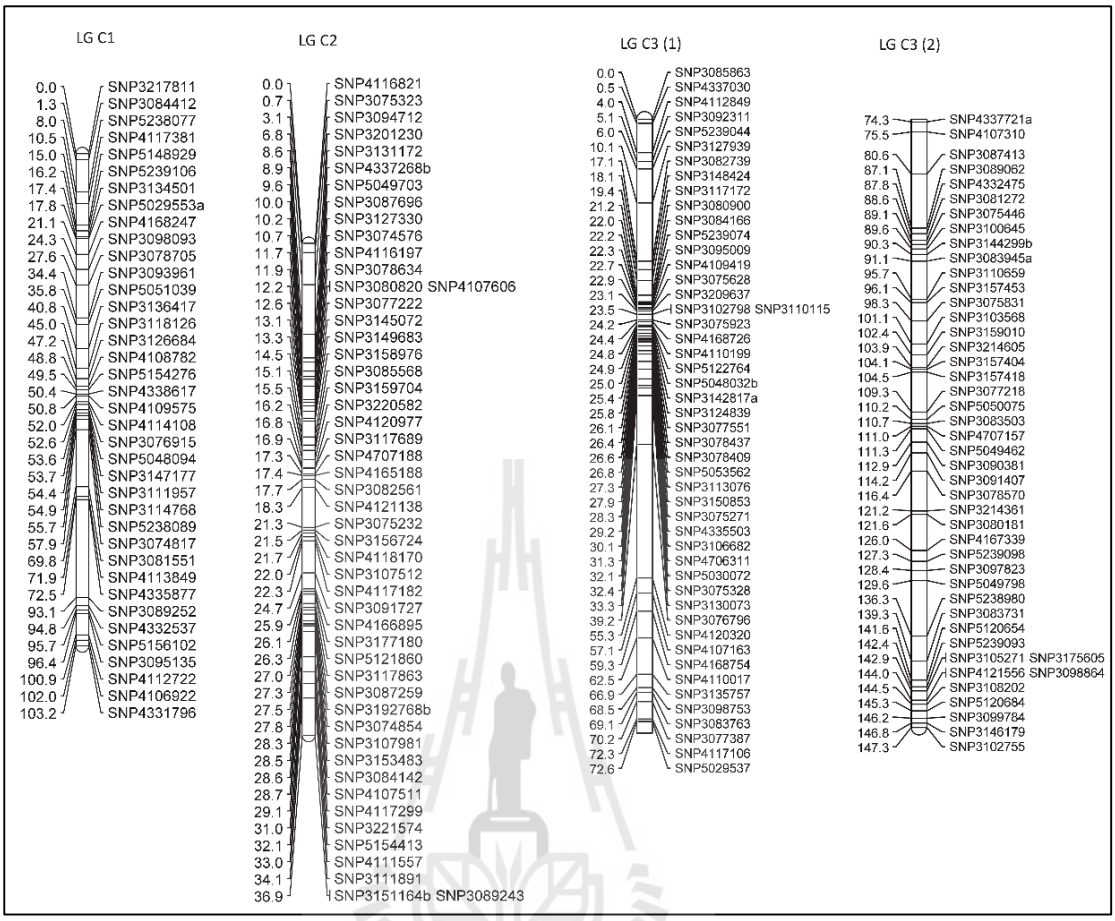

Figure 1 (Continued) The genetic linkage framework map (Map 1) of BC_1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

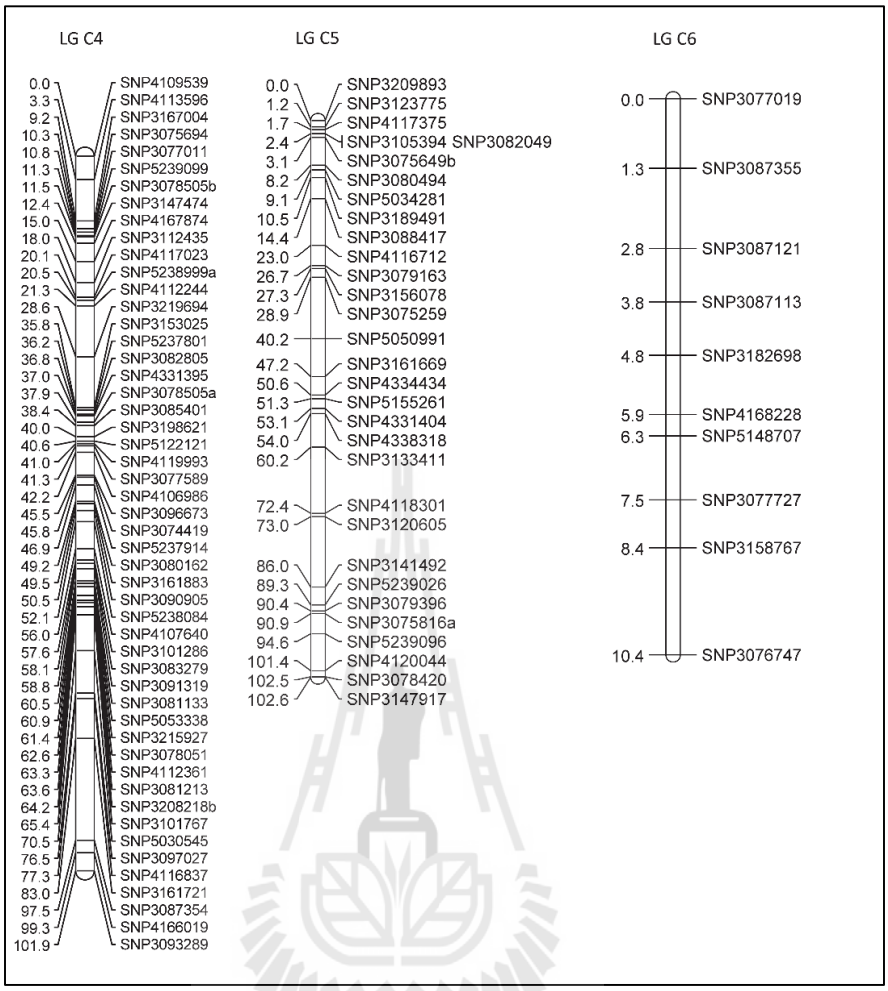

Figure 1 (Continued) The genetic linkage framework map (Map 1) of BC_1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

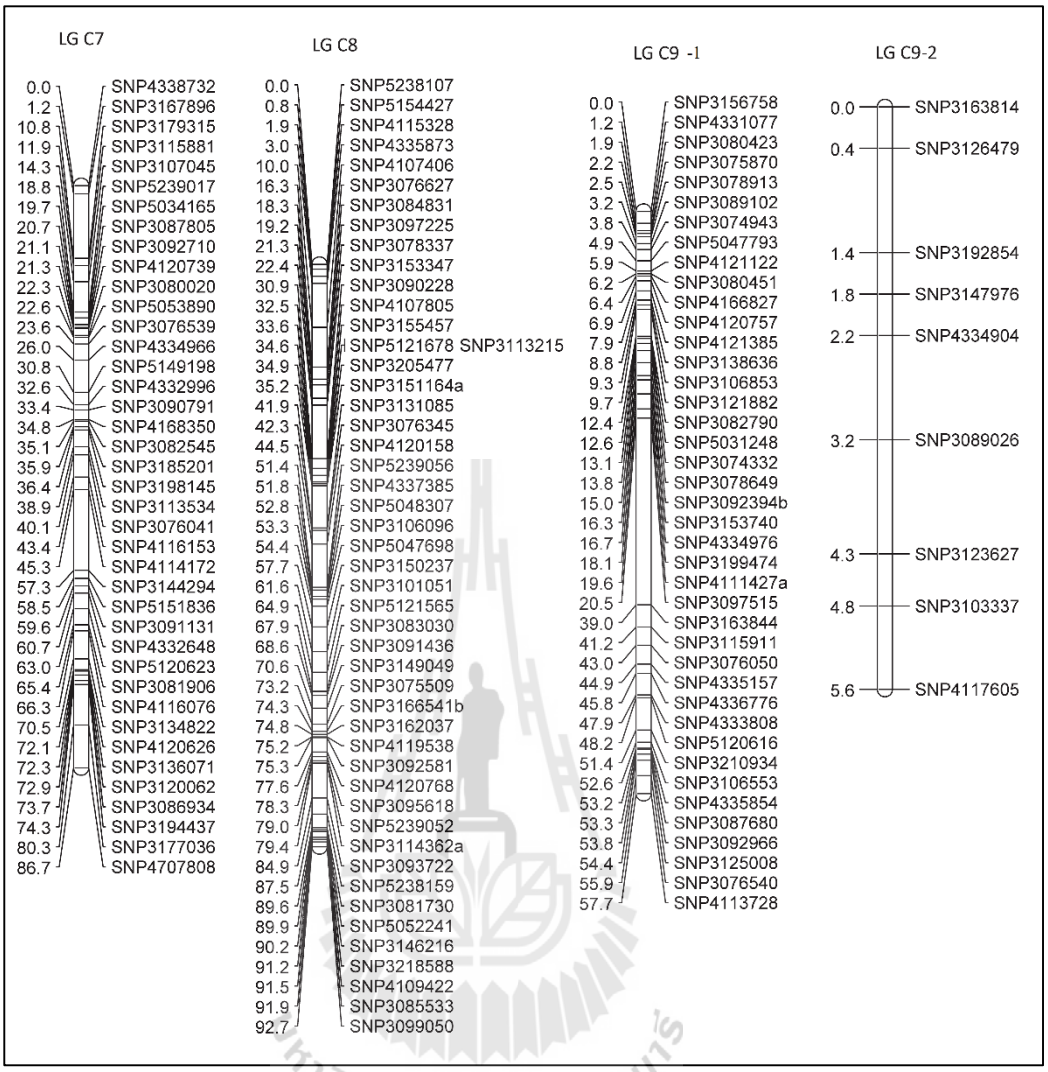

Figure 1 (Continued) The genetic linkage framework map (Map 1) of BC1 population of 10-4438 × 10- Zi006 constructed by using high quality SNP markers.

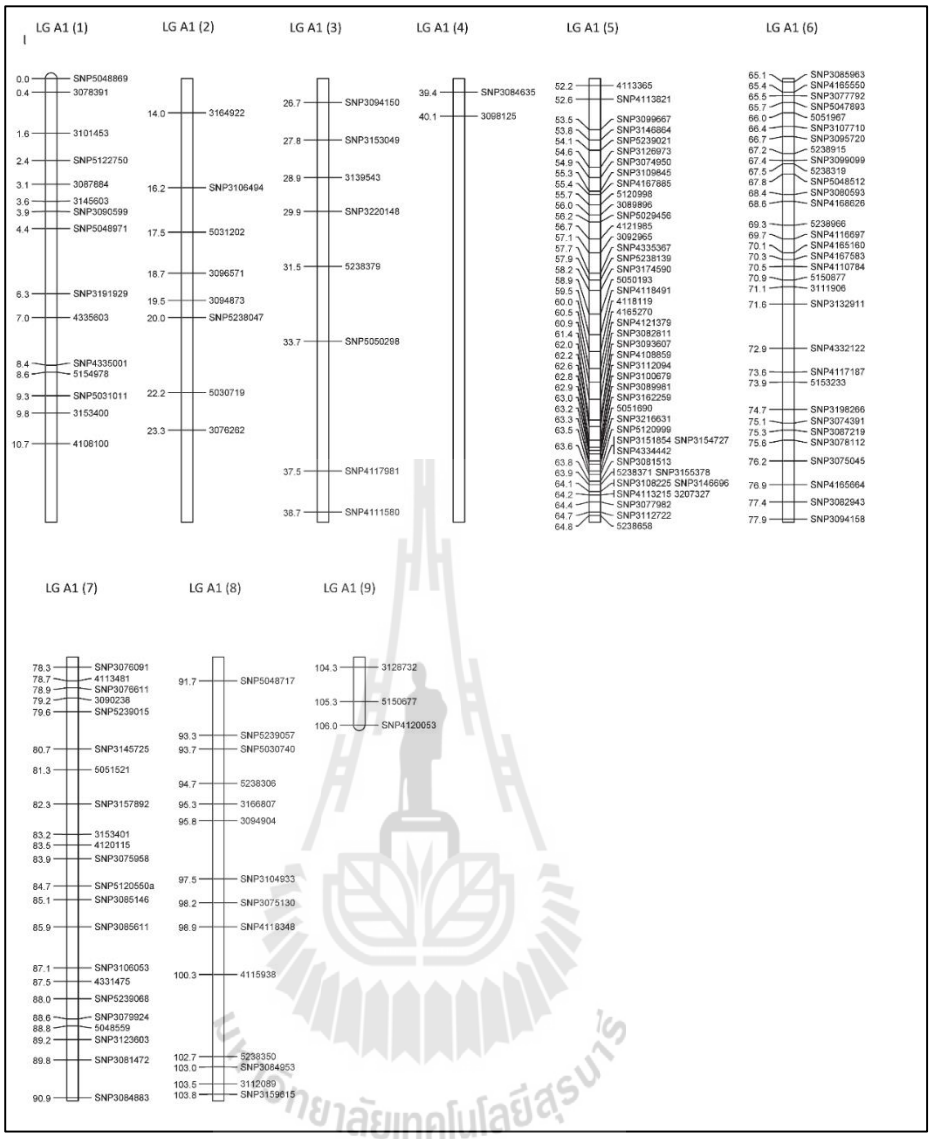

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

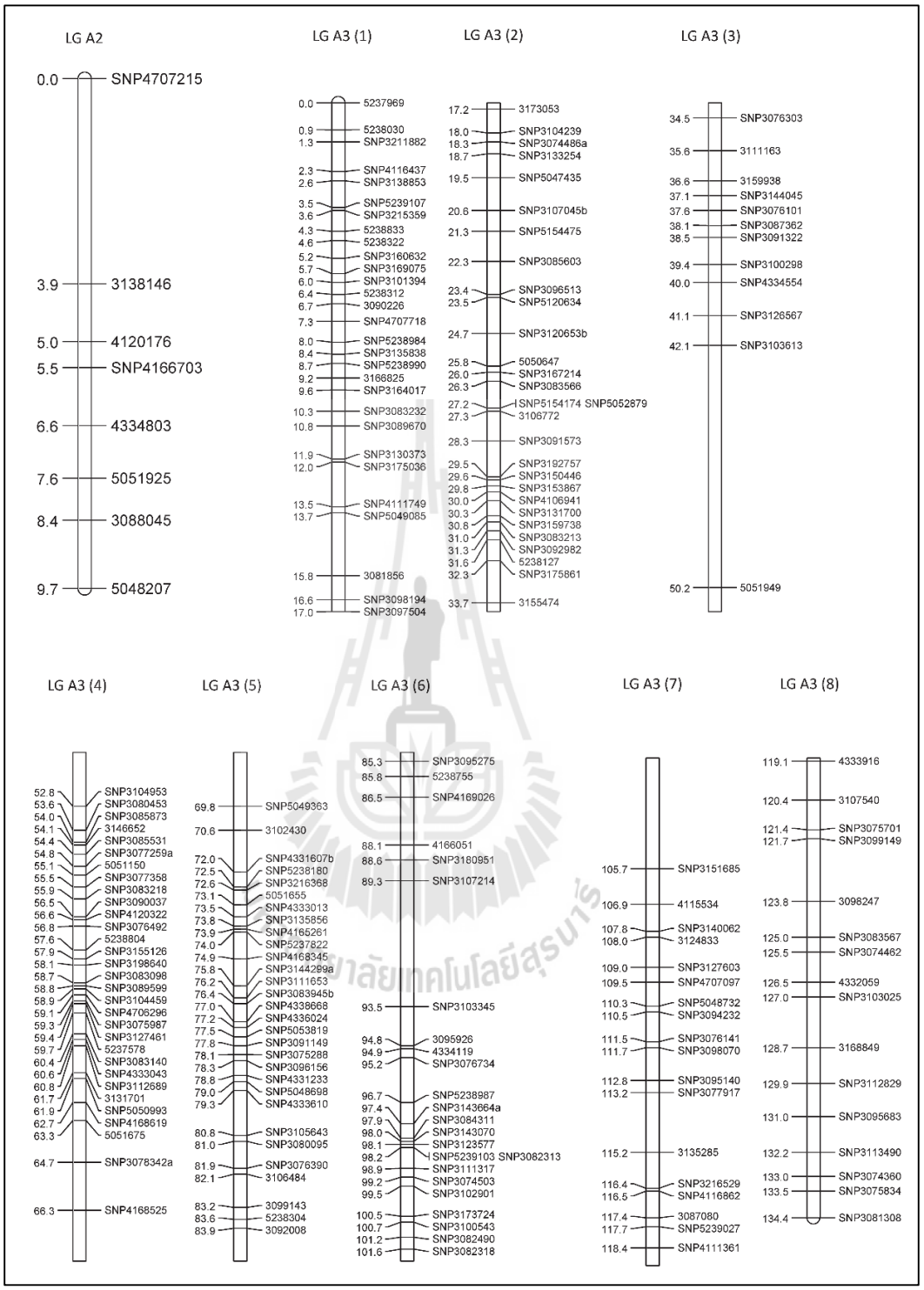

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

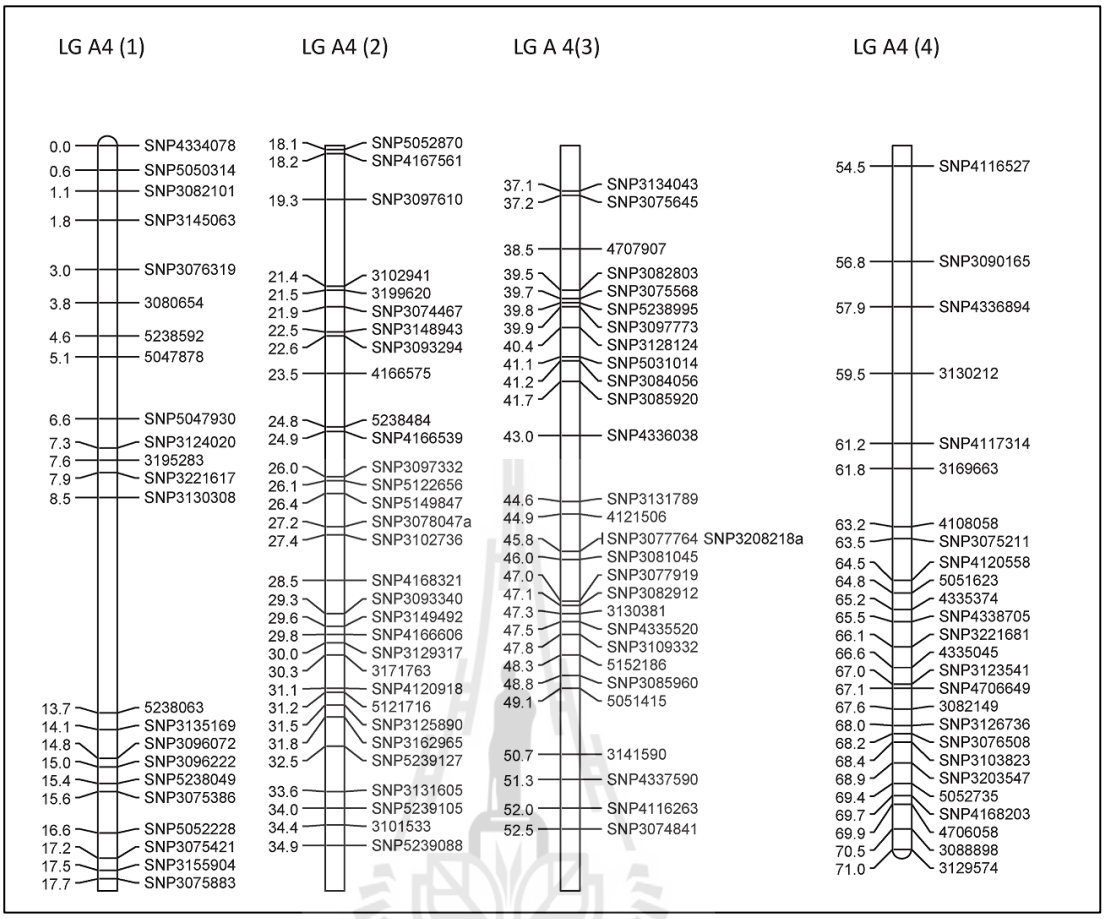

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

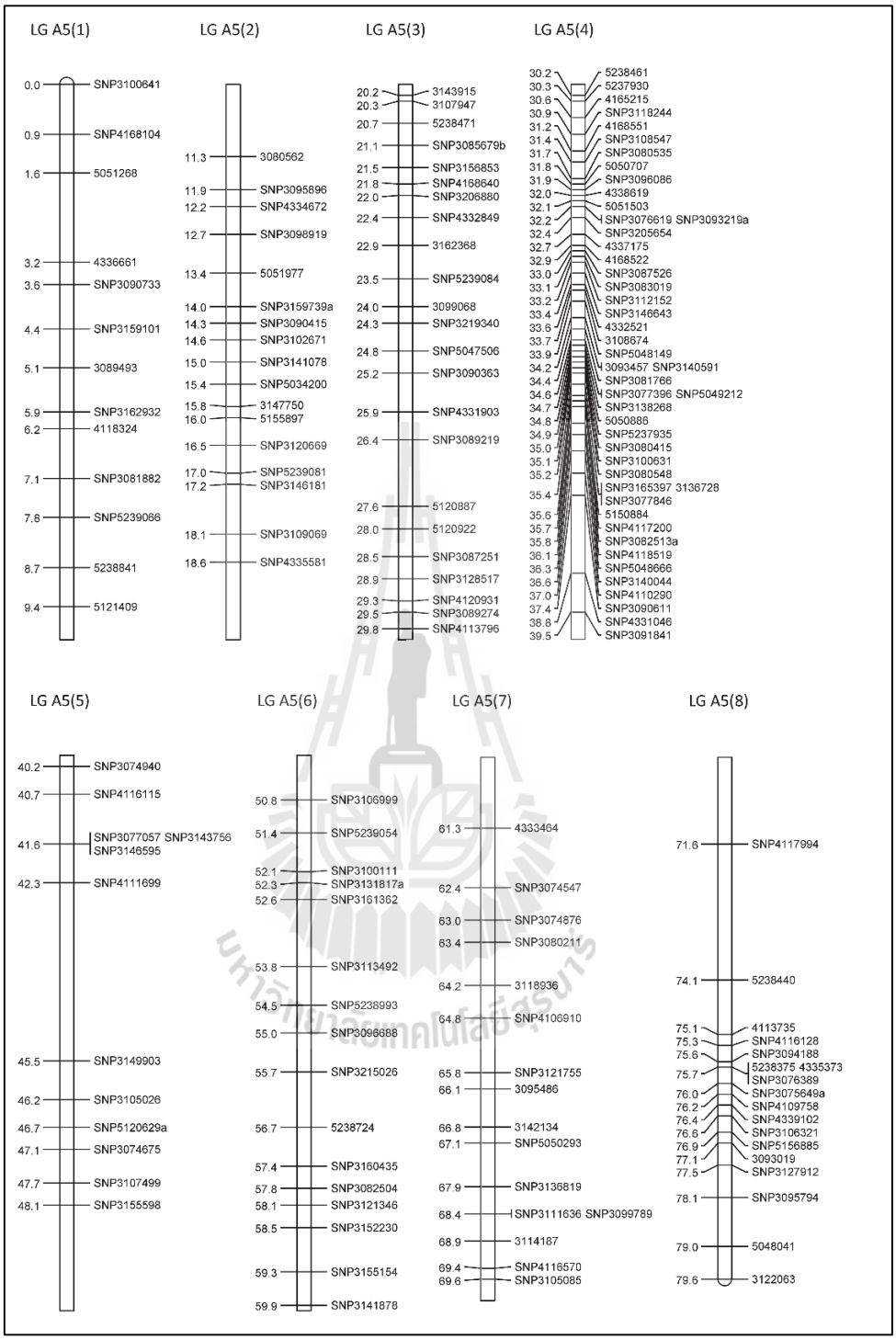

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

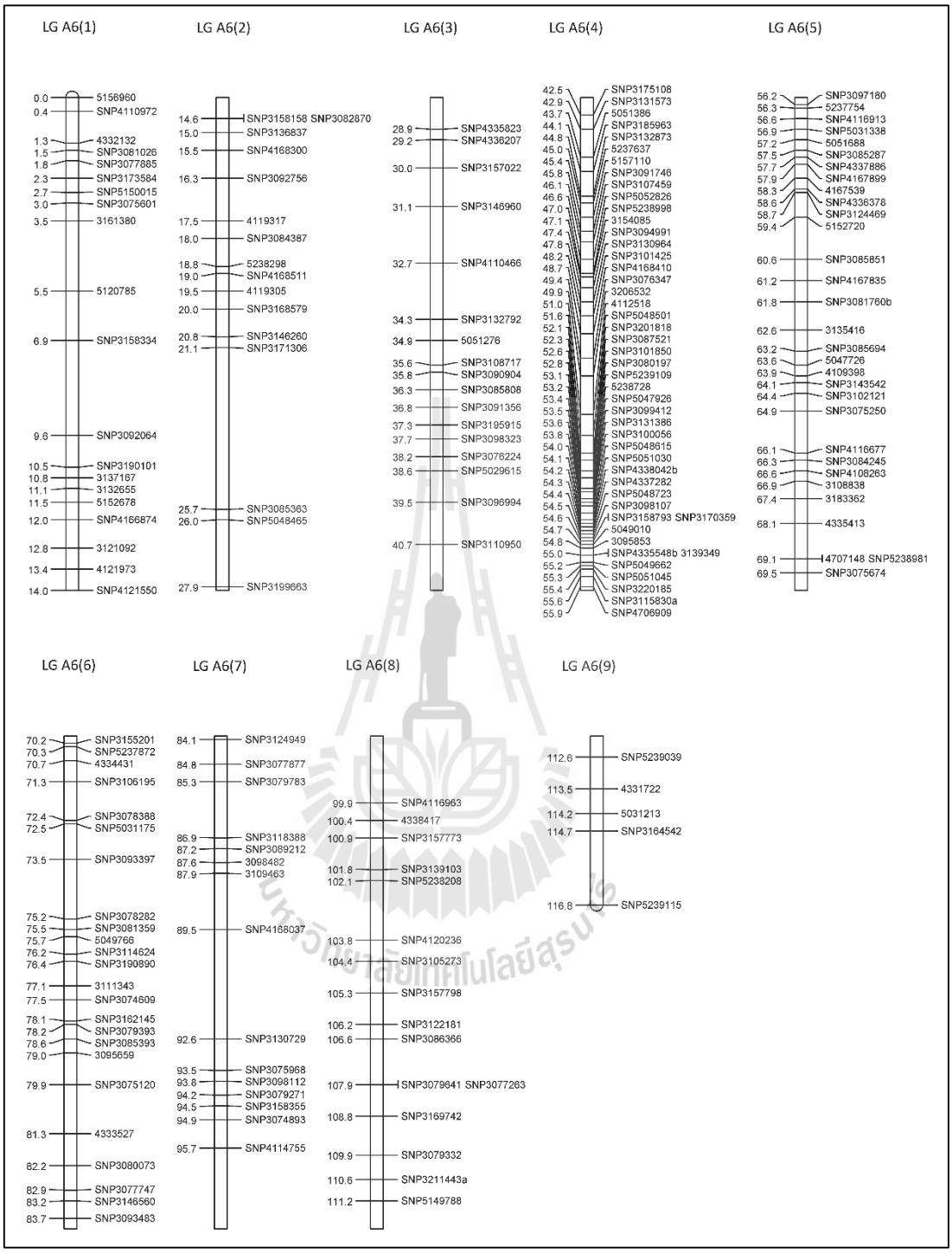

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

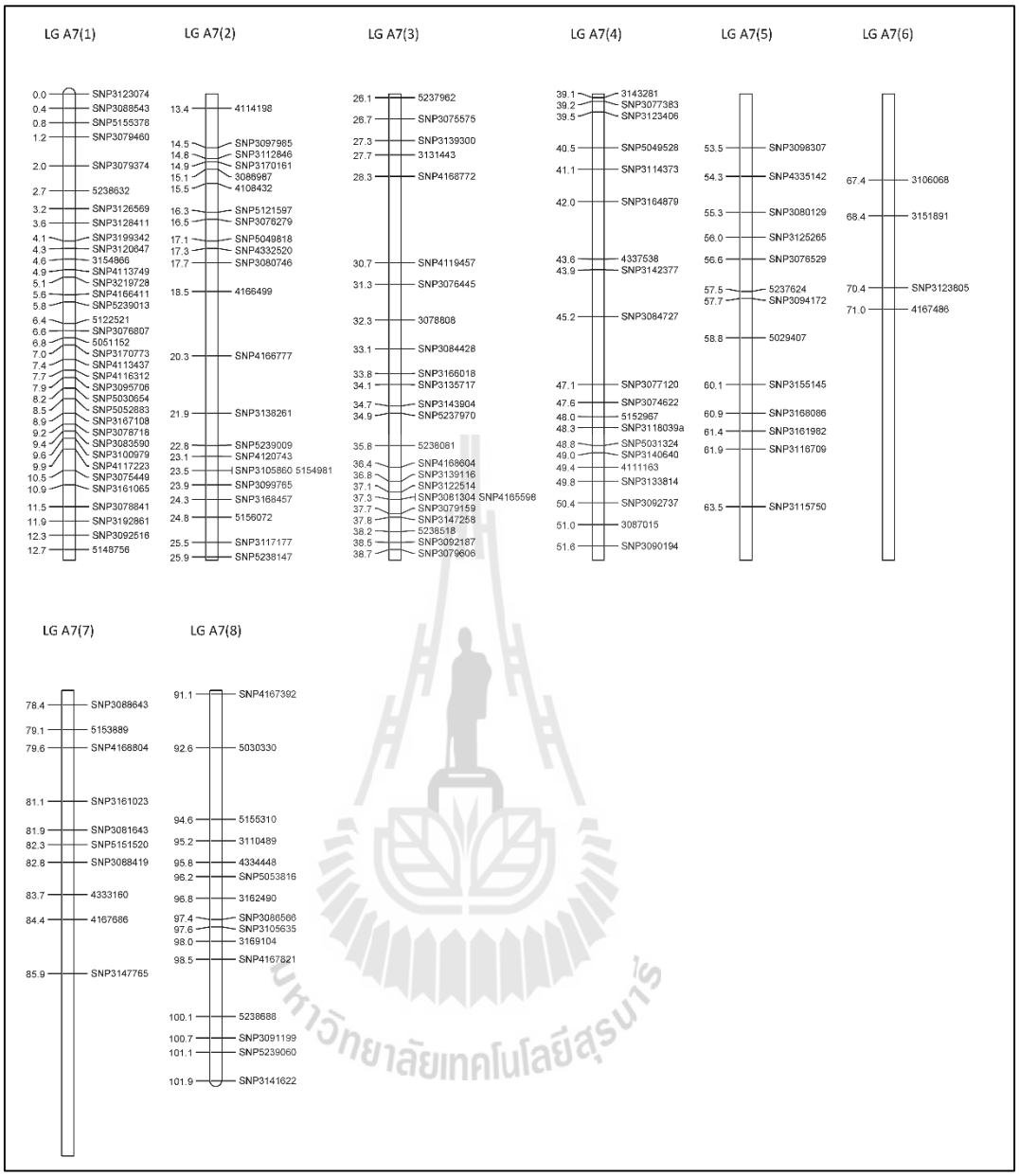

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

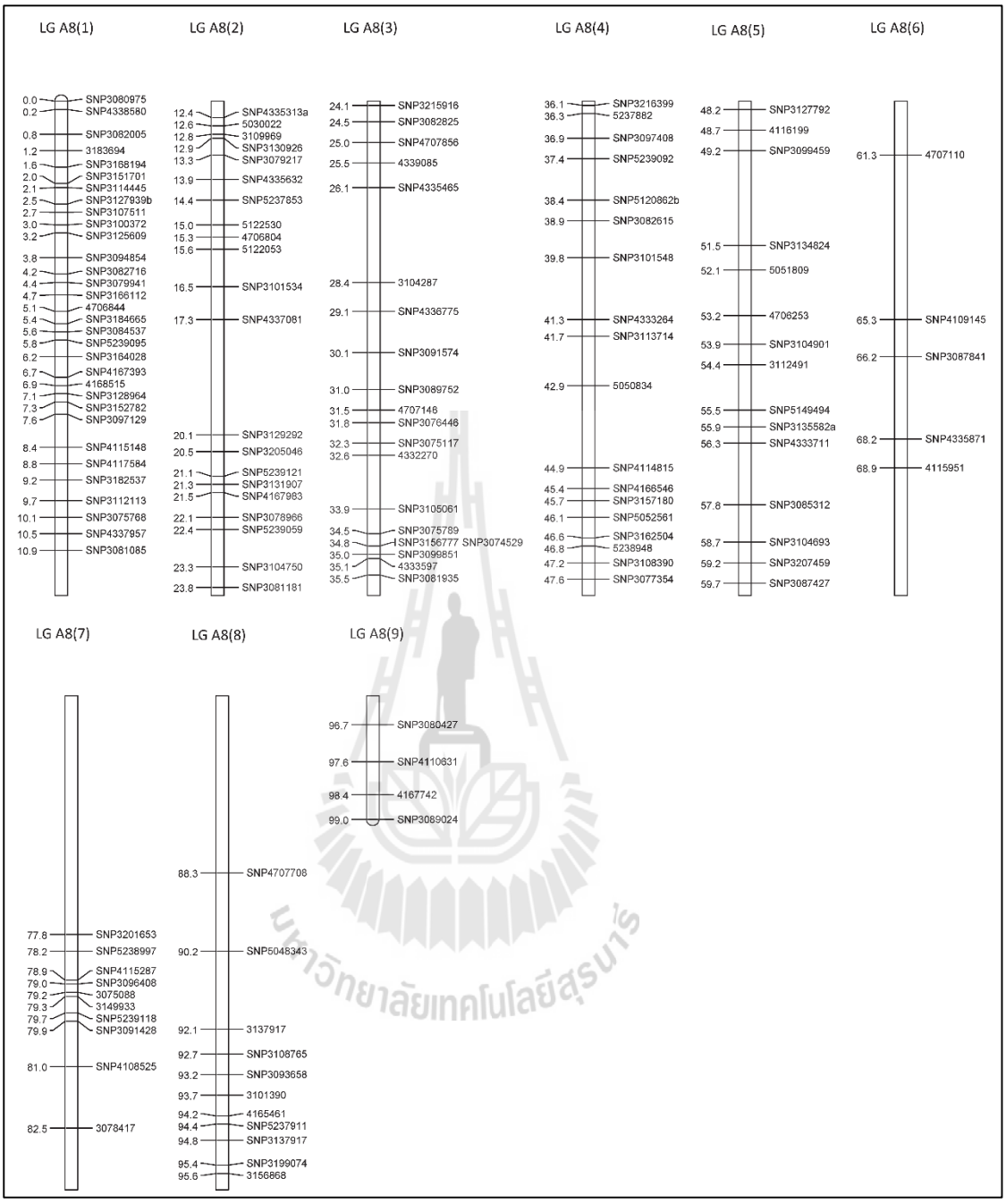

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

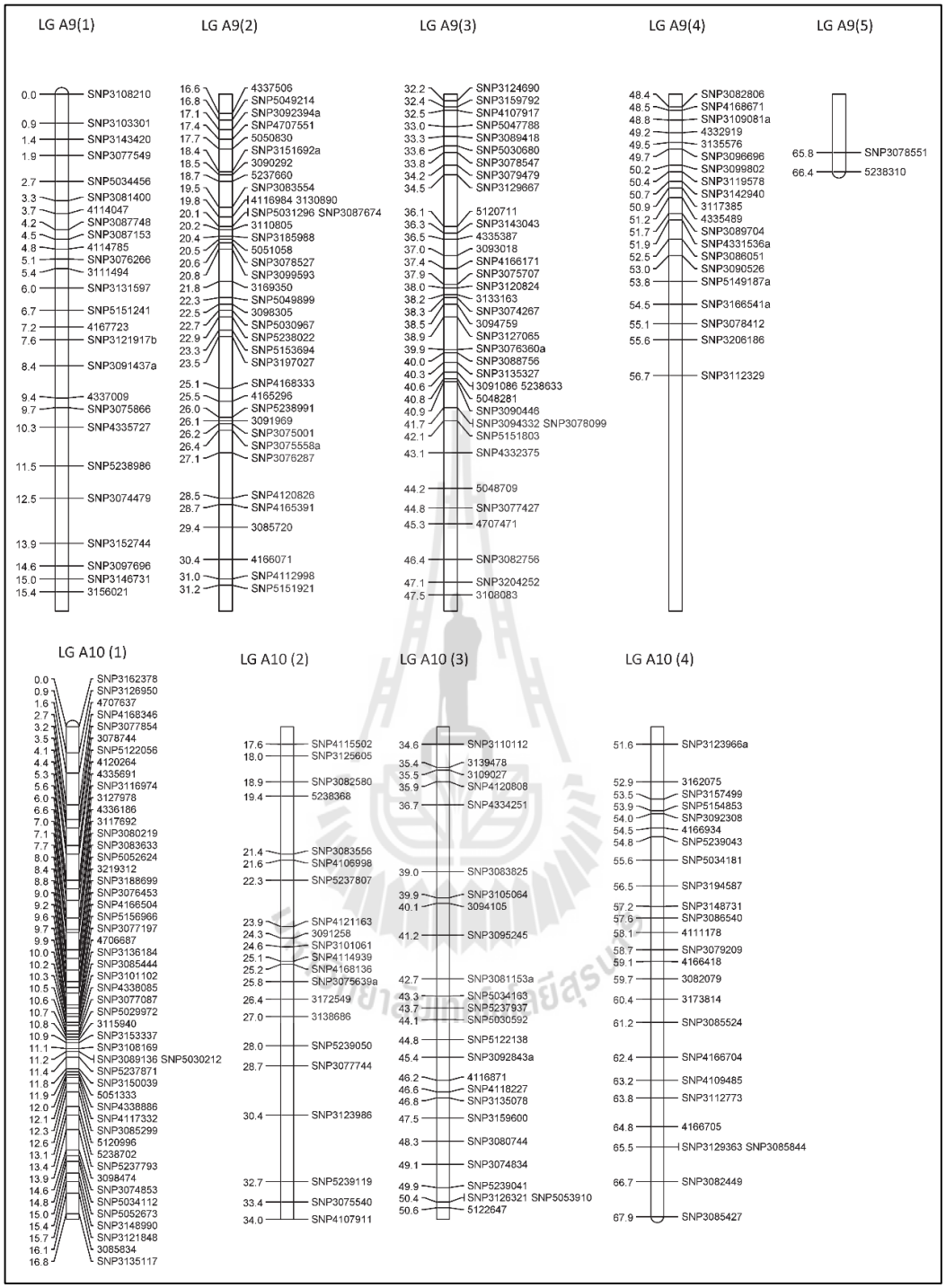

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

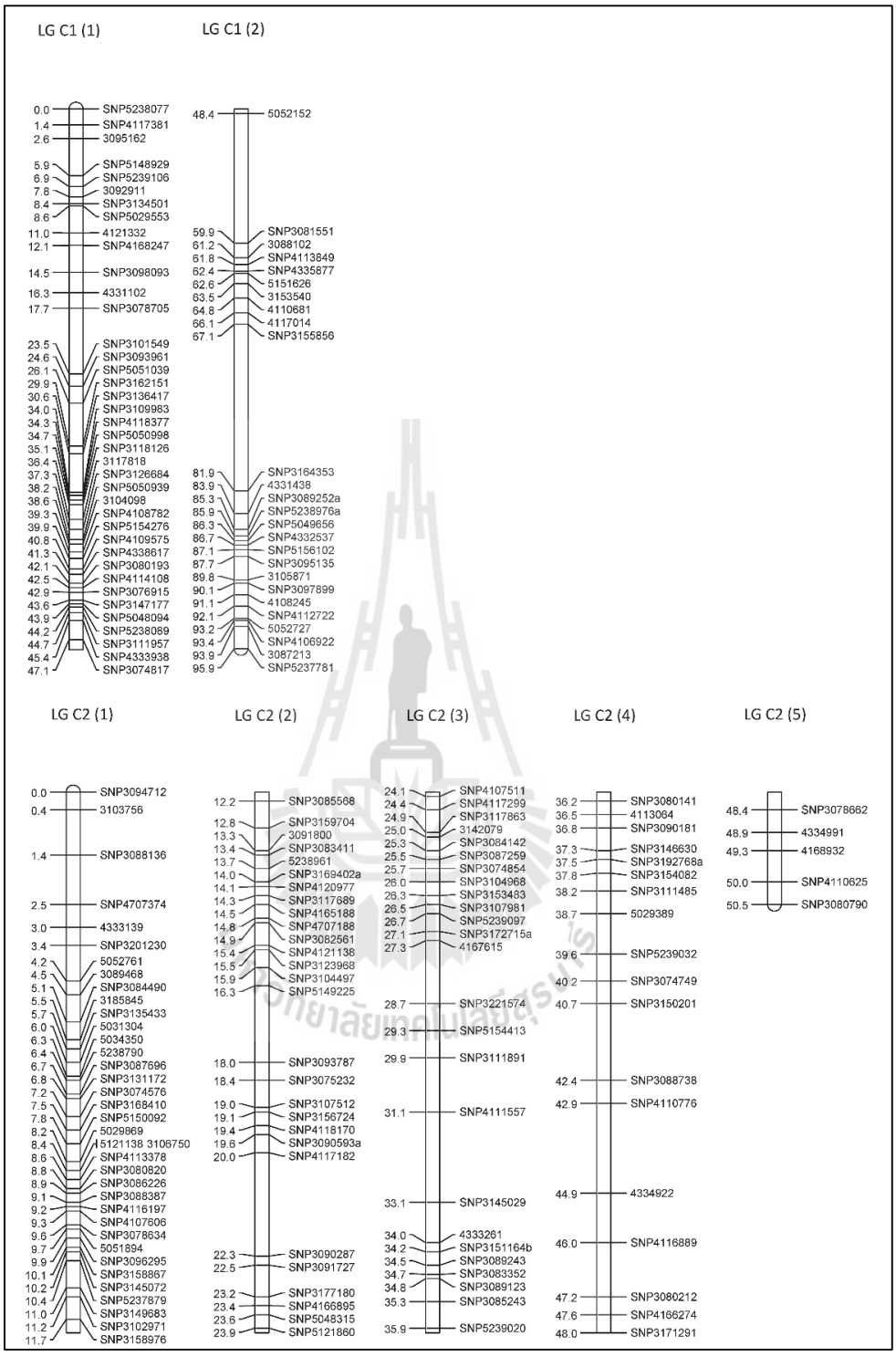

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

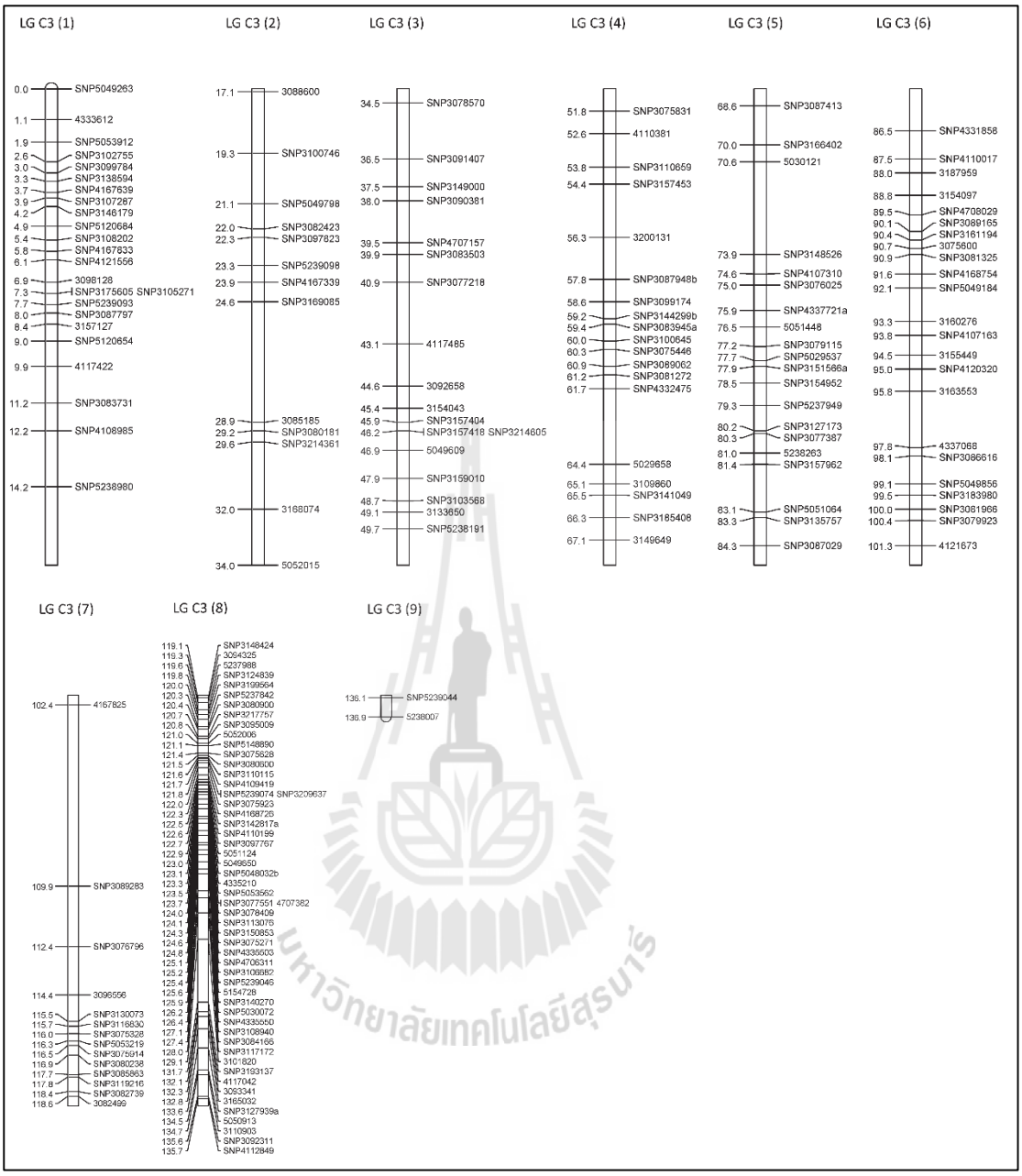

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

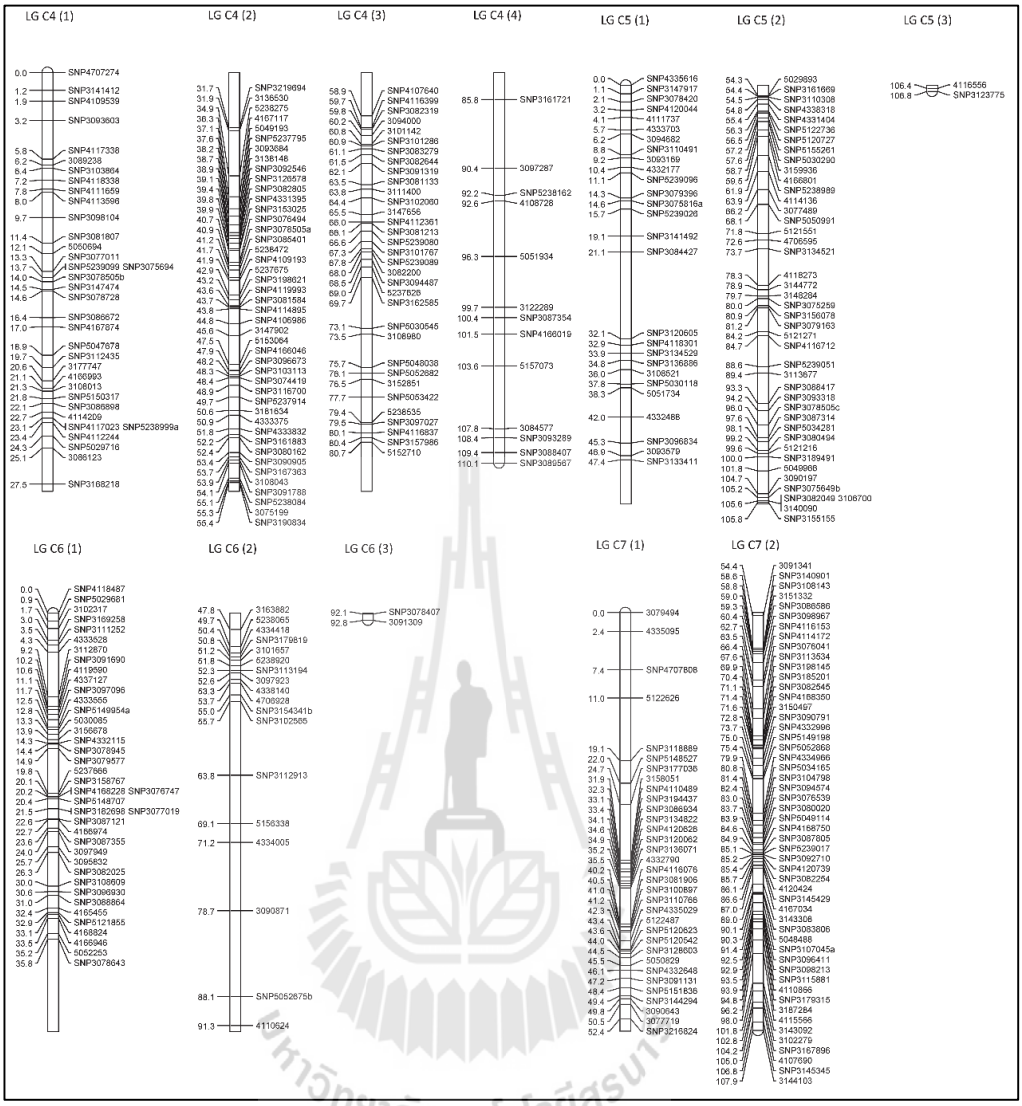

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

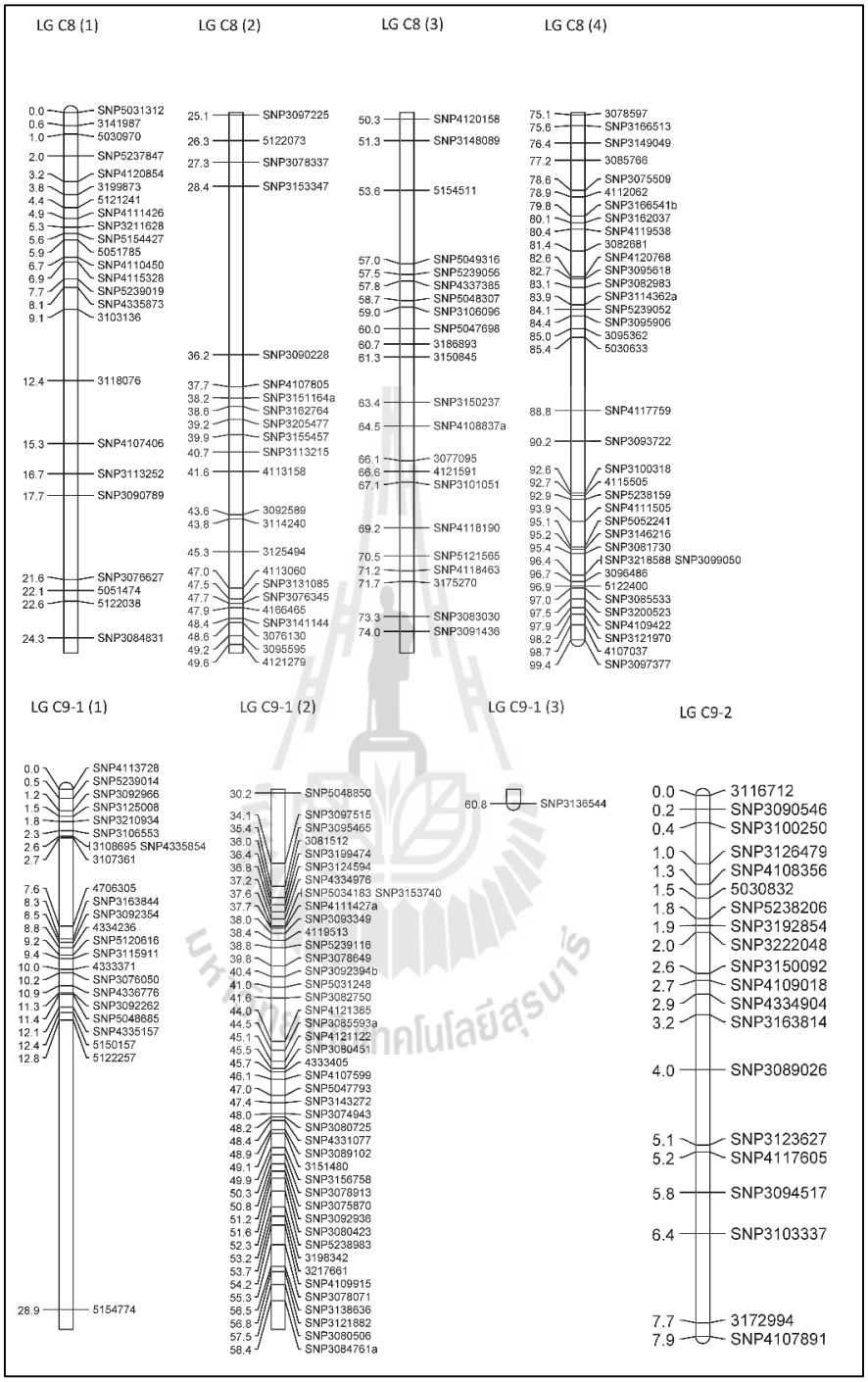

Figure 2 (Continued) The genetic linkage framework map (Map 2) of BC1 population of 10-4438 × 10- Zi006 constructed by attaching in sillco DArT markers and SNP markers (based on broad selection) into Map 1.

Table 6 The SNP marker information.

Chromosome	Linkage group	No. of markers				Aligned DArT -Seq markers with B. napus genome	% Aligned DArT -Seq markers with B. napus genome	Aligned DArT-Seq markers with B rapa and B oleracea genomes	% DArT-seq markers aligned with B. rapa and or B. oleracea	Transversions								Transitions			
		SNP	*in silico* DArT	total	% SNP Markers					A>C	C>A	G>T	T>G	A>T	T>A	C>G	G>C	A>G	G>A	C>T	T>C
A1	A1	229	407	636	36.01	192	83.84	197	86.03	13	12	9	10	14	13	8	10	25	39	40	36
A2	A2	73	267	340	21.47	68	93.15	63	86.30	5	4	5	2	5	3	2	4	14	12	11	6
A3	A3	313	554	867	36.10	279	89.14	282	90.10	18	14	6	12	26	23	15	14	48	47	40	50
A4	A4	179	327	506	35.38	157	87.71	162	90.50	10	11	17	10	10	13	9	5	20	26	24	24
A5	A5	276	514	790	34.94	237	85.87	244	88.41	17	13	14	17	11	16	11	15	40	41	38	43
A6	A6	293	470	763	38.40	261	89.08	255	87.03	11	16	13	18	20	21	9	19	43	44	44	35
A7	A7	215	294	509	42.24	200	93.02	200	93.02	12	13	12	11	11	20	7	8	38	25	28	30
A8	A8	216	336	552	39.13	190	87.96	204	94.44	16	16	14	11	19	11	5	6	26	31	30	31
A9	A9-1	135	216	351	38.46	118	87.41	125	92.59	7	7	6	5	12	2	5	10	26	18	14	23
	A9-2	43	130	173	24.86	37	86.05	35	81.40	3	3	3	1	2	3	3	4	8	7	2	4
A10	A10	229	340	569	40.25	204	89.08	201	87.77	11	7	15	16	17	19	11	12	32	32	19	38
C1	C1	58	107	165	35.15	46	79.31	41	70.69	4	3	6	8	3	4	6	3	7	2	5	7
C2	C2	220	366	586	37.54	191	86.82	189	85.91	11	16	10	17	10	21	4	9	29	28	32	33
C3	C3	234	403	637	36.73	212	90.60	215	91.88	20	11	12	8	14	14	7	11	38	33	34	32
C4	C4	148	248	396	37.37	124	83.78	133	89.86	6	4	7	6	10	11	12	7	17	25	21	22
C5	C5	68	130	198	34.34	65	95.59	62	91.18	8	6	3	2	6	5	6	1	6	5	11	9

Table 6 (Continued) The SNP marker information.

Chromosome	Linkage group	No. of markers				Aligned DArT -Seq markers with B. napus genome	% Aligned DArT -Seq markers with B. napus genome	Aligned DArT-Seq markers with B rapa and B oleracea genomes	% DArT-seq markers aligned with B. rapa and or B. oleracea	Transversions								Transitions			
		SNP	*in silico* DArT	total	% SNP Markers					A>C	C>A	G>T	T>G	A>T	T>A	C>G	G>C	A>G	G>A	C>T	T>C
C6	C6	69	123	192	35.94	63	91.30	66	95.65	3	3	4	2	3	4	2	2	12	7	15	12
C7	C7	89	111	200	44.50	81	91.01	77	86.52	3	4	4	7	6	4	4	3	15	11	10	18
C8	C8	135	290	425	31.76	122	90.37	110	81.48	6	6	10	8	14	4	6	7	24	10	22	18
C9	C9-1	90	114	204	44.12	80	88.89	80	88.89	4	4	3	5	5	4	3	3	19	15	13	12
	C9-2	46	108	154	29.87	41	89.13	42	91.30	0	2	3	4	2	4	5	1	7	4	4	10
Total		3358	5855	9213	36.45					188	175	176	180	220	219	140	154	494	462	457	493

APPENDIX C

THE ALIGNMENT OF QTL WITH PHYSICAL MAP

Table 7 The well alignment of QTL regions for seed oil content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
flanking marker	SNP3080095	A03	92.78	chrA03	16.17
qOC-a03-1	SNP3105643	A03	93.88	chrA03	16.08
qOC-a03-1	4109879	A03	93.88	chrA03	16.08
qOC-a03-1	3079403	A03	93.88	chrA03	16.09
flanking marker	SNP4331233	A03	96.01	chrA03	13.12
	The difference		**3.24**		**3.05**
flanking marker	4167985	A03	99.48	chrA03	16.20
qOC-a03-2	SNP4333610	A03	101.62	chrA03	10.32
flanking marker	SNP3096156	A03	102.87	chrA03	4.66
	The difference		**3.39**		**11.55**
flanking marker	SNP3115424	A05	79.37	chrA05	10.08
qOC-a05-1	SNP3093219b	A05	83.77		
qOC-a05-1	SNP3093219a	A05	83.77		
flanking marker	SNP4166042	A05	85.97	chrA05	9.82
	The difference		**6.60**		**0.26**
flanking marker	SNP3076653	A06	158.99	chrA06	16.88
qOC-a06-2	5051149	A06	165.59	chrA06	16.08
qOC-a06-2	5050530	A06	165.59	chrA06	21.35
qOC-a06-2	3077122	A06	165.59	chrA06	16.91

Table 7 (Continued) The well alignment of QTL regions for seed oil content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
qOC-a06-2	3179696	A06	165.59	chrA06	17.47
qOC-a06-2	5051465	A06	165.59	chrA06	21.28
qOC-a06-2	SNP3097180	A06	165.59	chrA06	16.52
qOC-a06-2	4115313	A06	165.59	chrA06	16.56
qOC-a06-2	3128537	A06	165.59	chrA06	16.13
qOC-a06-2	SNP4116913	A06	165.59	chrA06	16.75
qOC-a06-2	5051688	A06	165.59	chrA06	16.31
qOC-a06-2	SNP3085287	A06	168.89	chrA06	19.18
qOC-a06-2	SNP4167899	A06	168.89	chrA06	19.24
qOC-a06-2	3103617	A06	168.89	chrA06	18.03
qOC-a06-2	SNP5149388	A06	168.89	chrA06	19.17
qOC-a06-2	4109864	A06	168.89	chrA06	19.33
qOC-a06-2	3094928	A06	168.89	chrA06	17.66
flanking marker	3111491	A06	169.99	chrA06	19.64
	The difference		**11.00**		**2.76**
flanking marker	SNP5239050	A10	92.09	chrA10	12.14
qOC-a10	5049887	A10	93.19	chrA10	12.14
qOC-a10	SNP4114939	A10	93.19	chrA10	12.14
flanking marker	4331484	A10	94.93	chrA10	11.81
	The difference		**2.85**		**0.33**

Table 7 (Continued) The well alignment of QTL regions for seed oil content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
flanking marker	3154062	C9-1	14.25	chrC09	32.50
qOC-c09-1	4120318	C9-1	15.38	chrC09	32.47
qOC-c09-1	3086906	C9-1	15.38	chrC09	32.33
qOC-c09-1	3089543	C9-1	15.38	chrC09	32.33
qOC-c09-1	SNP3154333	C9-1	15.38	chrC09	32.32
qOC-c09-1	3115576	C9-1	15.38	chrC09	32.33
qOC-c09-1	3118839	C9-1	15.38	chrC09	32.65
qOC-c09-1	SNP3094992	C9-1	15.38	chrC09	32.52
qOC-c09-1	3103548	C9-1	15.38	chrC09	32.63
qOC-c09-1	3129393	C9-1	15.38	chrC09	32.33
qOC-c09-1	SNP3078913	C9-1	15.38	chrC09	32.53
qOC-c09-1	3124314	C9-1	15.38	chrC09	32.33
qOC-c09-1	SNP3089102	C9-1	16.51	chrC09	31.93
qOC-c09-1	4168582	C9-1	16.51	chrC09	31.93
qOC-c09-1	3151480	C9-1	17.617	chrC09	32.07
flanking marker	3099341	C9-1	19.816	chrC09	21.06
The difference			**5.56**		**11.44**
flanking marker	3085977	C9-1	19.82	chrC09	21.06
qOC-c09-2	SNP3080093	C9-1	20.92	chrC09	18.90
qOC-c09-2	SNP5047793	C9-1	20.92	chrC09	18.93
flanking marker	SNP3177178	C9-1	28.61	chrC09	19.34
The difference			**8.80**		**1.72**

Table 8 The well alignment of QTL regions for leaf anthocyanin content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
flanking marker	5151778	A03	57.68	chrA03	1.42
qAC-a03-1	5237576	A03	58.81	chrA03	1.59
qAC-a03-1	SNP4114190	A03	58.81	chrA03	7.97
qAC-a03-1	SNP4332603	A03	58.81	chrA03	8.44
qAC-a03-1	SNP3075237	A03	58.81	chrA03	14.38
qAC-a03-1	SNP3102901	A03	58.81	chrA03	14.43
flanking marker	SNP3082313	A03	61.01	chrA03	14.53
The difference			**3.33**		**13.11**
flanking marker	SNP3076734	A03	73.11	chrA03	15.24
qAC-a03-2	SNP3103345	A03	74.21	chrC03	15.31
qAC-a03-2	3136049	A03	74.21	chrA03	15.45
flanking marker	SNP3107214	A03	79.73	chrA03	15.47
The difference			**6.62**		**0.23**
flanking marker	SNP5237858	A03	177.28	chrC03	23.08
qAC-a03-3	5238848	A03	179.48	chrC03	23.26
qAC-a03-3	SNP3155126	A03	179.48	chrC03	23.29
flanking marker	SNP3076492	A03	181.68	chrC03	23.29
The difference			**4.40**		**0.21**
flanking marker	SNP5237861	A03	184.98	chrA03	9.24
qAC-a03-4	SNP3140178b	A03	188.28	chrC03	23.29
qAC-a03-4	3157737	A03	188.28	chrA03	23.38
qAC-a03-4	SNP4120322	A03	188.28	chrA03	23.38
flanking marker	5238716	A03	190.48	chrA03	23.78

Table 8 (Continued) The well alignment of QTL regions for leaf anthocyanin content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
The difference			**5.50**		**14.54**
flanking marker	SNP3085702	A04	63.92	chrA04	7.76
qAC-a04	SNP4707217	A04	66.32	chrA04	11.51
flanking marker	5237639	A04	68.91	chrA04	5.49
The difference			**4.99**		**2.28**
flanking marker	SNP3215026	A05	151.18	chrA05	20.84
qAC-a05-1	5238724	A05	152.31		
flanking marker	4337489	A05	155.60	chrA05	20.98
The difference			**4.41**		**0.14**
flanking marker	SNP4336633	A05	161.11	chrA05	21.04
qAC-a05-2	4333464	A05	163.07	chrA05	21.08
qAC-a05-2	SNP3077039	A05	164.40	chrA05	21.24
qAC-a05-2	5050697	A05	164.40	chrA05	21.21
qAC-a05-2	3091018	A05	164.40	chrA05	21.25
qAC-a05-2	5237744	A05	164.40	chrA05	21.24
qAC-a05-2	5051950	A05	164.40	chrA05	21.21
qAC-a05-2	SNP3074547	A05	164.40	chrA05	21.27
qAC-a05-2	SNP3095990	A05	164.40	chrA05	21.25
qAC-a05-2	5238560	A05	164.40	chrA05	21.27
qAC-a05-2	3143854	A05	164.40	chrA05	21.22
qAC-a05-2	3122297	A05	164.40	chrA05	21.21
flanking marker	SNP3074876	A05	165.50	chrA05	21.30
The difference			**4.40**		**0.25**

Table 8 (Continued) The well alignment of QTL regions for leaf anthocyanin content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
flanking marker	4116488	A05	166.60	chrA05	21.46
qAC-a05-3	3095486	A05	167.70	chrA05	21.47
qAC-a05-3	3118936	A05	168.80	chrA05	21.47
qAC-a05-3	SNP3121755	A05	171.00	chrA05	21.47
flanking marker	SNP5050293	A05	172.10	chrA05	21.96
The difference			**5.50**		**0.50**
flanking marker	SNP3077747	A06	207.96	chrC07	26.58
qAC-a06-1	SNP3093483	A06	209.06	chrC07	26.06
flanking marker	SNP3079783	A06	212.36	chrC07	27.10
The difference			**4.40**		**0.52**
flanking marker	SNP3078542	A06	220.06	chrC07	32.46
qAC-a06-2	SNP3080968	A06	222.26	chrC07	32.98
qAC-a06-2	SNP3079271	A06	222.26	chrC07	32.97
flanking marker	SNP3074893	A06	224.49	chrC07	32.67
The difference			**4.43**		**0.21**

Table 8 (Continued) The well alignment of QTL regions for leaf anthocyanin content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
flanking marker	SNP3111891	C02	65.26	chrC02	40.70
qAC-c02	SNP3221574	C02	66.36	chrC02	39.78
qAC-c02	3111907	C02	66.36	chrC02	39.78
qAC-c02	4116553	C02	66.36	chrC02	39.68
qAC-c02	3121237	C02	66.36	chrC02	40.70
qAC-c02	3104970	C02	69.19	chrC02	39.26
qAC-c02	SNP3153483	C02	69.19	chrC02	39.00
qAC-c02	SNP3107981	C02	69.19	chrC02	39.35
qAC-c02	3150148	C02	69.19	chrC02	39.00
qAC-c02	3090878	C02	69.19	chrC02	39.35
qAC-c02	3105321	C02	69.19	chrC02	39.25
qAC-c02	3165081	C02	69.19	chrC02	39.35
flanking marker	SNP3152789	C02	70.92	chrAnn_random	32.24
The difference			**5.66**		**8.46**
flanking marker	3152400	C04	60.39	chrC04	41.37
qAC-c04-2	SNP3162585	C04	63.69	chrA05	13.21
flanking marker	SNP5239089	C04	64.87	chrC04	41.39
The difference			**4.48**		**0.02**

Table 9 The well alignment of QTL regions for leaf chlorophyll content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
flanking marker	SNP3085963	A01	176.46	chrA01	15.98
qCC-a01	SNP3151854	A01	178.66	chrA01	14.61
flanking marker	3161450	A01	181.96	chrA01	14.23
The difference			**5.50**		**1.75**
flanking marker	5238833	A03	297.69	chrA03	2.88
qCC-a03-2	5051666	A03	299.89	chrA03	0.93
flanking marker	SNP3148583	A03	302.09	chrA03	5.84
The difference			**4.40**		**2.96**
flanking marker	SNP3076653	A06	158.99	chrA06	16.88
qCC-a06	5051149	A06	165.59	chrA06	16.08
qCC-a06	5050530	A06	165.59	chrA06	21.35
qCC-a06	3077122	A06	165.59	chrA06	16.91
qCC-a06	3179696	A06	165.59	chrA06	17.47
qCC-a06	5051465	A06	165.59	chrA06	21.28
qCC-a06	SNP3097180	A06	165.59	chrA06	16.52
qCC-a06	4115313	A06	165.59	chrA06	16.56
qCC-a06	3128537	A06	165.59	chrA06	16.13
qCC-a06	SNP4116913	A06	165.59	chrA06	16.75
qCC-a06	5051688	A06	165.59	chrA06	16.31
flanking marker	SNP4337886	A06	167.79	chrAnn_random	18.34
The difference			**8.80**		**1.46**
flanking marker	3178737	A07	131.29	chrA07	17.63
qCC-a07	SNP3081064	A07	133.50	chrA07	17.71

Table 9 (Continued) The well alignment of QTL regions for leaf chlorophyll content with *B. napus* genome.

QTL Designation	Renamed marker ID	Linkage group	Genetic position (cM)	Chromosome	Physical position (Mb)
qCC-a07	SNP3092737	A07	133.50	chrA07	17.71
qCC-a07	5051741	A07	136.93	chrA07	17.74
qCC-a07	3155716	A07	136.93	chrA07	17.92
qCC-a07	SNP3100378	A07	136.93	chrA07	17.88
flanking marker	SNP3080129	A07	141.23	chrA07	18.43
The difference			**9.94**		**0.80**
flanking marker	SNP3111957	C01	28.96	chrC01	11.30
qCC-c01	SNP3121803	C01	30.06	chrC01	10.97
qCC-c01	4707474	C01	30.06	chrC01	10.89
qCC-c01	3085076	C01	30.06	chrC01	10.97
qCC-c01	SNP3076915	C01	30.06	chrC01	10.89
qCC-c01	3105326	C01	30.06	chrC01	11.11
flanking marker	SNP3080193	C01	32.25	chrC01	10.82
The difference			**3.29**		**0.48**
flanking marker	SNP3122036	C03	189.73	chrC03	49.14
qCC-c03	SNP4119438	C03	191.93	chrC03	50.10
qCC-c03	4338805	C03	191.93	chrC03	50.15
qCC-c03	SNP3113076	C03	191.93	chrC03	50.11
qCC-c03	4335370	C03	191.93	chrC03	50.11
qCC-c03	SNP3108940	C03	191.93	chrC03	49.97
flanking marker	SNP5053562	C03	193.03	chrC03	50.58
The difference			**3.30**		**1.44**

BIOGRAPHY

Mr. Dai Wendong was born on 15, Sep. 1975 in Jinsha county, Guizhou province, P. R. China. He holds a BA degree in Agronomy from Southwest Agriculture University and a M.S degree in Agricultural extension from Guizhou University. Mr. Dai Wendong is currently a Ph.D. candidate in Crop Science under the supervision of Dr. Teerayoot Girdthai in the School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Thailand.