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EGE UNIVERSITY

Graduate School of Applied and Natural Science



MAPPING QTLs FOR IMPORTANT AGRONOMIC TRAITS ON EARLY SEGMENT OF WHITE CABBAGE

MSc THESIS

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EGE UNIVERSITY
Graduate School of Applied and Natural Science

**MAPPING QTLS FOR IMPORTANT AGRONOMIC
TRAITS ON EARLY SEGMENT OF WHITE
CABBAGE**

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2020

Here we certify that this thesis entitled “**Mapping QTLs for Important Agronomic Traits on Early Segment of White Cabbage**” conducted, prepared and submitted for the degree of Master of Science by Katarina JOVIĆ has been recommended for acceptance and approval for oral defence as it complies both with the “Ege University Graduate School policies” and “Ege University Graduate School of Natural and Applied Sciences' rules and regulations”. This study is fully adequate, in scope and quality, as a thesis for the degree of Master of Science and the Candidate was found successful and the thesis was approved by the thesis committee unanimity/majority voting on 25 /09 / 2020.

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25/09/2020



Katarina JOVIĆ

ÖZET**ERKENCİ BAŞ LAHANADA BAZI ÖNEMLİ AGRONOMİK
ÖZELLİKLERİN QTL HARİTALANMASI**

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Yüksek Lisans Tezi, Tohumluk Bilimi ve Teknolojisi Anabilim Dalı

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Bu tez çalışmasında, baş lahananın (*Brassica oleracea* var. *capitata*) 12 agronomik özelliğin QTL haritalanması amaçlanmıştır. Tarla denemeleri, Macaristan'daki Syngenta (Ócsa) Deneysel Yetiştirme İstasyonunda Şubat ve Haziran 2020 arasındaki dönemde gerçekleştirilmiştir. Haritalama çalışmaları, ebeveyn olarak iki double haploid hat (P1 ve P2) kullanılması ile oluşturulan F2 popülasyonunda gerçekleştirilmiştir. P1 ve P2 hatları Syngenta baş lahana germplasm koleksiyonundan temin edilmiş olup, F2 popülasyonunda 910 bitkide agronomik değerlendirmeler gerçekleştirilmiştir.

Bağlantı haritası oluşturmak ve QTL analizi gerçekleştirmek için R x 64 3.6.2 programında (R/qtl paketi), 369 polimorfik SNP işaretleyicisi kullanılmıştır. Interval Mapping (IM) analizi, Haley Knott regresyonu kullanılarak 0.001 tahmini genotipleme hataları ile gerçekleştirilmiştir. Composite Interval Mapping (CIM) analizi, R/qtl fonksiyonu ile yapılmış ve çoklu QTL analiz modeli araştırılmıştır. LOD skor eşik değeri olarak permütasyon testi $N = 1000$ ve 1.5 LOD güven aralığı kullanılmıştır. R/qtl programında Bağlantı Haritaları oluşturularak QTL'ler harita üzerinde gösterilmiştir.

Toplamda, 61 putative QTL bölgesi belirlenmiş olup, bunlardan 7'sinin major QTL olduğu tespit edilmiştir. Majr QTL'ler, LOD skoru > 5 olan ve %10'dan fazla fenotipik varyasyonu açıklananlardır. Bu 7 major QTL'den 6 tanesi 2. kromozomda 14.99 cM - 25.01 cM aralığında belirlenmiştir. StLen.2.2,

HdLen.2.1, HdRat.2.1, HdDen.2.2, Col.2.1 ve Vig.2.1'e en yakın işaretleyici AXO16308, Ant.7.5'e ise en yakın işaretleyici AXO14084 olarak belirlenmiştir. Baş şekli (HH / HW oranı) ile ilgili genlerinin epistatik etkide olduğu saptanmıştır.

Anahtar kelimeler: QTL analizi, *Brassica oleracea* var. *capitata*, Composite Interval Mapping, Bağlantı Haritası, R/qtl.



ABSTRACT**MAPPING QTLS FOR IMPORTANT AGRONOMIC TRAITS ON
EARLY SEGMENT OF WHITE CABBAGE**

JOVIĆ, Katarina

Master Thesis, Department of Seed Science and Technology

Supervisors: Prof. Dr. Hülya İLBİ

Co-supervisor: Dr. Zsolt GALLI

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This thesis research describes a QTL analyses for 12 main agronomic traits of the white cabbage (*Brassica oleracea* var. *capitata*). Field experiments were performed in Syngenta (Ócsa) Experimental Breeding Station, in Hungary, in period between February and June 2020. Two parental doubled haploid lines (referred as P1 and P2) were used to develop the F2 mapping population. P1 and P2 lines were acquired from the Syngenta white cabbage germplasm collection. Nine hundred ten plants of F2 population were used for data collection.

Altogether 13 traits were measured, even though just 12 traits were included into the thesis project. Due to the Syngenta Company's Privacy Policy, the results of Core length QTL analyses were not shown in the thesis.

Three hundred sixty-nine polymorphic SNP markers were used to build Linkage Map and perform QTL analyses in R x64 3.6.2 program, R/qtl package. Initial interval mapping was performed with estimated genotyping errors of 0.001 using Haley Knott regression. Composite Interval Mapping was performed with "cim" R/qtl function. Multiple QTL analyses model was searched using an iterative process. LOD score thresholds were obtained using permutation test N=1000. 1.5 LOD confidence intervals were used. Linkage Map View package for R/qtl software was used to produce linkage group maps with QTLs and their confidence intervals marked on the map.

In total, 61 putative QTL regions were detected and among them seven major QTLs. Major QTLs were those with LOD score > 5 and more than 10% phenotypic variation explained. Out of these seven major QTLs, six were found within the same interval (14.99 cM – 25.01 cM) and on the same chromosome (chromosome 2). The marker closest to the StLen.2.2, HdLen.2.1, HdRat.2.1, HdDen.2.2, Col.2.1 and Vig.2.1 was AXO16308, while marker closest to the Ant.7.5 was AXO14084. Epistasis was found in case of Head shape (HH/HW ratio).

Keywords: QTL analyses, *Brassica oleracea* var. *capitata*, Composite Interval Mapping, Linkage Map, R/qtl.



PREFACE

Considering the fact that cabbage contains vitamin C concentration in almost the same amount as orange and other citrus fruits, it is no surprise that popularity of cabbage as great healthy food source has grown in the last decade. High amount of vitamin K, glucosinolates, carotenoids and other phytochemicals, as well as minerals such as potassium have shown the importance of this plant as an antioxidant and anticarcinogenic source. Being a very adaptable plant and a strong survivor, cabbage has managed to thrive in many farming and climate conditions all over the world. In last ten years, cabbage has seen steady increase in production. This trend should lead to 80 million tonnes of cabbage produced worldwide by the end of 2025.

Cabbage is in top five produced vegetables globally. Various plant breeding programs are aiming to improve the genetics and performance of this crop to keep their variety competitive and desirable on the market. Marker assisted selection is an important tool in varietal development. Quantitative Trait Loci analyses can give us an insight and the right strategy in the breeding program. However, most of the QTL analyses of the cabbage and the rest of the Brassica family were performed with disease, bolting or abiotic stress resistance. Agronomical traits were not as researched as previously mentioned topics. Physical traits and plant architecture can also help in plant's ability to resist to certain pest or type of abiotic stress. Agronomical traits such as the colour, head weight, head shape etc. are highly important since they determine the popularity of the variety on the market. While biotic and abiotic resistance are important, it is the general plant appearance that will be noticed by the final consumers.

Mapping the important QTLs for agronomically important traits could help cabbage breeders guide their programs toward stronger and healthier plants that would be able to stay competitive in ever growing cabbage variety market.

IZMIR

25 / 09 / 2020

Katarina JOVIĆ

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1. INTRODUCTION

1.1 Crop Description

Cabbage (*Brassica oleracea* var. *capitata*) is a cultivar that belongs to the *Brassica oleracea* species, which is one of the most diverse species in *Cruciferae* (*Brassicaceae*) family and it contains more than 338 genera and around 3 709 species (Downey, 2016).

The *Cruciferae* family group includes three diploid species: *B. rapa* (AA, $2n = 2x = 20$), *B. nigra* (BB, $2n = 2x = 16$) and *B. oleracea* (CC, $2n = 2x = 18$). Also, there are three amphiploid species: *B. juncea* (AABB, $2n = 4x = 36$), *B. napus* (AACC, $2n = 4x = 38$) and *B. carinata* (BBCC, $2n = 4x = 34$). The three diploid species, among which is cabbage, contain the basic chromosome sets, while the amphiploid species contain hybridized and naturally doubled combinations of the three diploid species. Relationship between these species is known as U's triangle (Figure 1.1). The genome size of the diploid *Brassica* is between 529 and 696 Mb. The genome sizes of the allopolyploids is between 1068 and 1284 Mb (Wang et al., 2012).

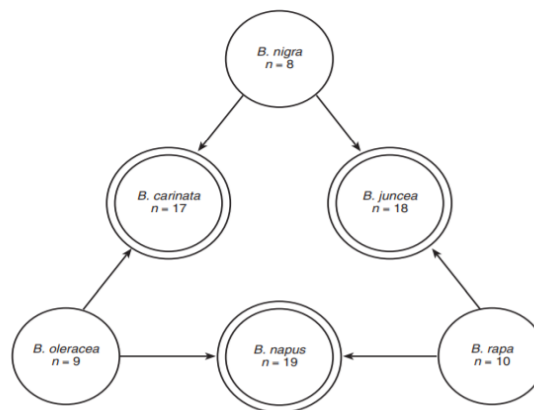


Figure 1.1. The triangle of U; U, 1935 (Dixon, 2006).

This vast diversity and flexibility of *Brassica* genetics have proven to be a good tool in domestication of the species in Neolithic era. Since then, it was spread throughout the world, which enabled cultivation of these plants in the

Ancient Greek, Roman, and Chinese civilizations (Dixon, 2006). It has been proven by historical evidences that modern day head cabbage cultivars are descended from their wild non-heading ancestor that has originated from the eastern Mediterranean and Asia Minor. North European countries and the Baltic Sea region, as well as the Mediterranean coast, are traditionally viewed as the centre of origin and domestication of cabbage (Balkaya et al., 2005).

Human selection has modified a common wild mustard plant into several crop cultivars by targeting a different organ of the plant (Figure 1.2). While Kale is a product of breeding for leaves along the stem, Brussels sprouts is that of breeding for enlarged buds. Broccoli and cauliflower are products of breeding for inflorescences. And cabbage came after breeding for leaves surrounding the terminal bud. Cabbage is divided into three main cultivar groups: white cabbage, red cabbage, and savoy (Ordás and Cartea, 2008).

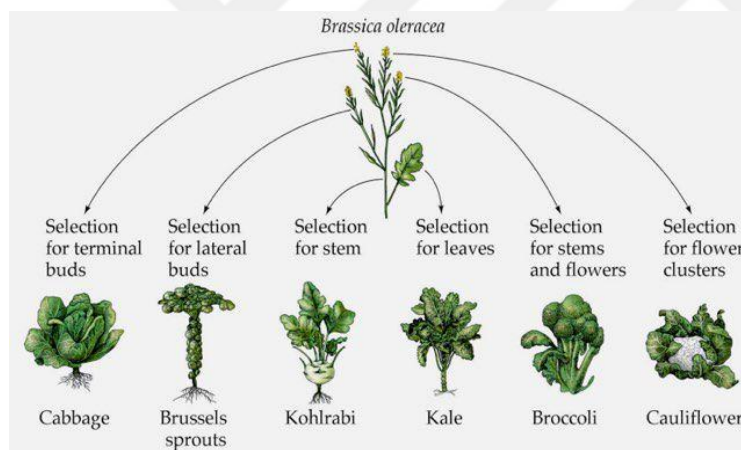


Figure 1.2. *Brassica oleracea* diversity.

(<https://www.vox.com/xpress/2014/8/6/5974989/kale-cauliflower-cabbage-broccoli-same-plant>)

Cabbage is a biennial plant. Cabbage growth cycle starts with a short stem which terminates into a large bud composed of a dense, overlapping, smooth leaves, a part known as the “head” (Figure 1.3). Large fleshy leaves surround the head in a rosette. This is a harvestable part, grown as an annual crop, even though cabbage is biennial plant (OECD and Downey, 2016). Heads are around 40-60 cm tall and their weight range is from 0.5 to 6.0 kg. Heads are round to oblate in shape. Leaves are light to dark green in colour, and since the inner leaves are not

exposed to light, they are whitish in colour (Dixon, 2006). In the second year of growth, cabbage head is split open and the flower stalk grows to the height of 1.5-2.0 metres. Stalk's branches will be bearing flowers. Flowers are self-incompatible. In general, flowers are comprised of four sepals, four petals, six stamens, and two carpels. Flower pollination is carried out by insects (Balliu, 2015).

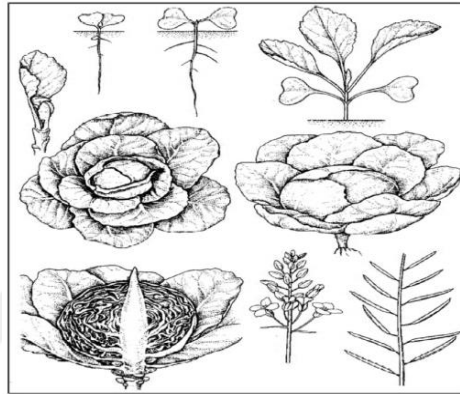


Figure 1.3. Cabbage growth cycle (Feller et al., 1995).

Even though it is adapted to wide variety of climates, in general, cabbage is a cool and humid climate crop, with the optimum growth temperature between 18°C and 20°C. It can tolerate strong frost, up to -3°C although more severe freezing temperatures can kill the crop or make it unmarketable. Water requirements range between 380 mm and 500 mm depending on a season. Cabbage can tolerate wide range of soil types, but well-drained, loamy soils with pH 5.5 - 6.5 are considered the best. Cabbage heavily uses nitrogen and potassium and thus requires frequent fertilization. Transplanting should be done during relatively cool weather. High temperatures or strong drying winds can delay recovery from transplanting stress. Distance between the plants depends on the cultivar. For the early white cabbage types, 30 – 40 cm distance between the plants and 60 – 75 cm distance between the rows is the most usual practice. Cabbage growing period varies between 90 days (for spring sown) and 200 days (for autumn-sown). This of course depends on climate conditions (Balliu, 2015; Kelley et al., 2009). Cabbage can be grown in early spring, so that it can be harvested in early summer, or it can be sown in mid-summer to be harvested in autumn, around September.

Regarding the chemical composition and nutritional value of the cabbage, carbohydrates are the main constituents of this vegetable since they are comprising almost 90% of the dry weight. On the average, one third of carbohydrates are dietary fibres and two thirds are low-molecular weight carbohydrates (Rokayya et al., 2013). Since Brassicas contain vitamins, carotenoids, and polyphenols, members of this family have both antioxidant and anticarcinogenic effects on human body (Podsędek, 2005). The white cabbage provides significant levels of: A, C, E and K vitamins, and other phytochemicals, such as glucosinolates or sulphur-containing compounds. It has been found that there is a significant variation in vitamins C and E levels among the different leaf layers of the white cabbage. It could be possible that these vitamins may play an important role in metabolic regulation and/or plant signalling (Nosek et al., 2011). Based on a cabbage chemical compounds review by (Podsędek, 2005), it has been found that vitamin C content varies in white cabbage somewhere between 18.8 mg/100g (Bahorun et al., 2004) and 46-47 mg/100g (Davey et al., 2000), which is quite good if we compare it to a more commonly known source of vitamin C, orange, that has around 58.3 mg/100g (Najwa and Azrina, A, 2016). In general, mineral content of cabbage is comprised of sodium, potassium, calcium, manganese, copper, iron, zinc, phosphorus. Potassium content is higher compared to the other minerals analysed (Table 1.1). Also, it is higher in cabbage than in other various species of Brassica vegetables. Common vitamins found in cabbage are vitamin A, vitamin C and folic acid, with vitamin C content being the highest (Table 1.1) (Ogbede et al., 2015).

Table 1.1. (left) Vitamin compositions of *Brassica oleraceae* var. *capitata* L. (right) Mineral compositions of *Brassica oleraceae* var. *capitata* L. (Ogbede et al., 2015)

		Parameters	Composition (mg/100g)
		Sodium	176±1.16
		Potassium	678±2.65
		Calcium	28.9±1.51
		Manganese	0.67±0.02
Parameters	Composition (per 100g)	Copper	0.05±0.003
Folic acid (µg)	86.30±0.69	Iron	2.15±0.10
Vitamin A (µg)	7.14±0.13	Zinc	2.11±0.04
Vitamin C (mg)	56.37±5.21	Phosphorus	26.92±0.47

The top five most produced vegetables in the world, in 2018, were: tomatoes, onions, cucumbers and gherkins, cabbages and other Brassicas, and eggplants (Omondi, 2018). Under normal rainfall conditions, cabbage yields reach 25 to 30 t/ha while in ideal, well irrigated conditions, with good quality crop management, could reach up to 85 t/ha (FAOSTAT, 2018). In the period of 10 years, between 2008 and 2018, cabbage has seen a steady increase in production (Figure 1.4). In 2018, cabbage and other Brassicas production was 69 million metric tonnes (FAOSTAT, 2018). The first world cabbage producer is China (33 million tonnes), followed by India (9 million tonnes) and Russia (2.5 million tonnes) (Figure 1.5.) (FAOSTAT, 2018). China is at the same time, the world's largest cabbage consumer. The largest cabbage consumption per capita was in Romania, South Korea and Ukraine. An increase in demand for cabbage worldwide is expected to continue. This trend could rise the market volume to 80 million tonnes by the end of 2025 (<https://www.researchandmarkets.com/>).



Figure 1.4. Cabbage world production and area harvested from 2008 to 2018 (FAOSTAT, 2018).

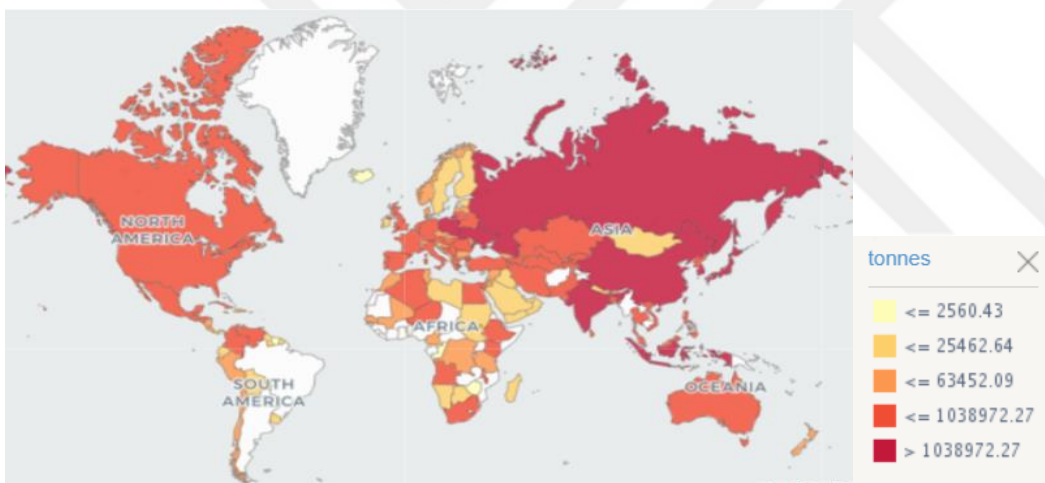


Figure 1.5. Cabbage world production in 2018 (FAOSTAT, 2018).

Breeding is an important tool for crop improvement and adaptation to a wider range of growing conditions. Main breeding goals in cabbage are, as well as in other crops, yield improvement, resistance to pests and diseases, and quality improvement (Fang et al., 2005). Recently, there has been an interest in improving the nutritional value and quality of cabbage and other Brassica species by changing the content of glucosinolates (Ordás and Cartea, 2008). Other breeding goals include: resistance to early bolting (since this can cause great economic losses), storability, uniformity (for easier mechanisation) and continuity (in order

to have all year round cabbage supply, we should have good early, mid and late varieties) (Fang et al., 2005). Natural self-incompatibility in cabbage as well as a wide range of available homozygous materials, makes cabbage a good breeding source for molecular assisted breeding and selection (Balliu, 2015). Generally, every breeding program will generate high amount of phenotypic data. Alongside with decreasing prices of genotyping, this has opened a door to deeper research of quantitatively inherited traits. The most of agronomically important traits have complex, quantitative inheritance model, which highlights the importance of QTL mapping that has become an important source of genetic improvement in breeding programs (Würschum, 2012). Classical breeding selection methods are costly and take time and it cannot be completely precise since it is based on phenotypic differences due to the environmental effects and cannot differentiate between heterozygous and homozygous alleles of traits. Current breeding programme in Brassicas is based on marker assisted selection (MAS), which helps breeders understand genetics behind characteristics as well improve their breeding strategies and results.

1.2 Thesis Goal

The main goal of this thesis project is mapping QTLs for important agronomic traits on early segment of white cabbage. The phenotypic traits such as stem height, outer leaf length, outer leaf width, leaf erectness, head weight, colour, head length, head width, core length, head shape, head density, anthocyanin discoloration, and vigour will be evaluated for QTL analysis. Then, molecular markers linked to these traits will be developed in this project. This will enable early selection via molecular markers for these traits in cabbage breeding programmes.

2. LITERATURE REVIEW

2.1 General QTL Analyses Overview

By definition, a trait whose phenotypic variation (that is due to genetics and/or environment) can be measured, is a quantitative trait. Even though the quantitative trait can sometimes consist of discrete values, it is usually continuous, such as height, weight etc. (Members of the Complex Trait Consortium, 2003). A polymorphic locus contains alleles that could affect the expression of this quantitative, continuous trait is called quantitative trait loci, or shortly QTL. A marker with statistical affiliation to a phenotypic trait's variation which is controlled by combined action of the alleles at multiple loci is usually considered to be a quantitative trait loci (Shimoyama et al., 2012). Genetic complexity that comes from allelic segregation at multiple loci, along with the environmental effects, could be the reason behind continuous phenotypic variation of the quantitative traits. However, the effect of these alleles on a trait could be very small so often, we can see different genotypes resulting in similar phenotypes. Also, because they are strongly affected by the environment, we can often observe same genotype giving rise to different phenotypes in different environments. Even though it is a challenging work to identify QTL, quantitative genetic variation is often behind susceptibility to certain diseases in both humans and plants, as well as yield and other agronomically important trait, thus it has future in further scientific research and development (Mackay, 2009). QTL analyses is a procedure concentrated on finding the connection between the quantitative trait in question and the marker alleles segregating in the population. Two main stages should be involved in analyses: mapping the markers and associating the trait with those markers. After the QTL analysis has been conducted, it should provide us with few fundamental answers. We should be able to tell where the QTLs are, what they do, how they interact and how they affect the trait. On a the wider scale, these answers should lead us to solutions in medicine (can they help us find the therapy) and in agriculture (can they assist us in breeding strategies) (Kearsey and Farquhar, 1998). There are five major steps in performing a good QTL mapping. First step is to develop an appropriate mapping population that will be derived from two parents which are a contrast in a desired trait. Second would be to

choose suitable molecular markers. Third would be a development of a high quality, saturated linkage map. Fourth step would be genotyping and phenotyping the population. Fifth step is using an appropriate statistical package to perform linkage analysis by using genotyping and phenotypic information collected before (Dhingani et al., 2015).

In order to connect which genes are responsible for which traits, phenotyping and genotyping must be conducted. Phenotyping is a technique that measures and describes plant characteristics, in association with available genetical information (Rahaman et al., 2015) while the purpose of genotyping is to determine the genetic make-up (genotype) of an individual by examining the individual's DNA sequence (Junaid et al., 2019). For this, building a mapping population is necessary. Mapping populations is a group of individuals derived from a cross between the parents differing in the traits that are to be studied. It is used to identify genetic loci that influence phenotypic variation and to determine the recombination distance between loci. Depending on the reproductive mode of the plant, mapping population type to be used will be determined (Schneider, 2005). The most common biparental cross populations used are: F₂ populations, backcross (BC) population, doubled haploids (DH), recombinant inbred lines (RIL), and near isogenic lines (NIL) (Xu et al., 2017). In an F₂ population, one inbred line is used as female parent, and it is crossed with another inbred line used as a male parent. The parental lines should be homozygous and different for a desired trait. The F₁ plants are then going to be selfed in order to develop F₂ seeds, which are later planted to develop an F₂ plant population. F₂ individual plants are genotyped for molecular markers and then phenotypically evaluated for the trait of interest (Zhang, 2012). The advantages of F₂ are rapid population development and ability to estimate both additive and dominant effects. Its disadvantages are in its limited recombination and temporary nature (not immortal population) (Xu et al., 2017). In a BC population, two homozygous inbred parental lines that differ for a specific trait are crossed with each other. Then, F₁ population will be crossed with one of the parents, which is also called the recurrent parent. Purpose is to introduce and investigate a specific genome fragment of interest (Schneider, 2005). However, the disadvantages of BC population are time required to develop the population, its temporary nature and

lack of ability to estimate the dominant effects (Xu et al., 2017). Doubled haploids are plants derived from a single pollen grain which was artificially doubled in order to get homozygous diploids. Since a DH plant has two identical homologs, the amount of recombination information is equal to a backcross. One of the advantages of a DH population used for molecular mapping is that all individuals are homozygous, which enables plant transfer between different laboratories and environments, which improves measure of environmental variation (Khan and Croser, 2004). DH populations are the most common choice in case of repetitive and multi-trait analysis of a genotype. It takes just one generation to achieve complete homozygosity, compared to inbreeding line development which takes many generations and much more time and cost. The downside of DH could be skewness of loci distribution, as well as segregation distortion (Prins et al., 2005). Recombinant inbred lines (RILs) are a group of strains that can be used to map quantitative trait loci. In order to get RILs, parental strains are crossed to create recombinants which will then be inbred to the level of isogenicity, resulting in a permanent source of trait mapping and analysis. To get good quality recombinant inbred lines, parent strains are selected based on phenotype, marker availability, and compatibility. At the end, lines are inbred to create genetically stable recombinant lines (Pollard, 2012). Near isogenic lines (NILs) are lines created by introgression of a single target locus from a donor variety into a recurrent (recipient) parent. In many cases, NILs are used to confirm previously found genetic loci that explain part of the variation observed in a certain trait of interest. Since many quantitative traits are controlled by multiple loci, each locus should be independently tested in a Mendelian fashion. This could show us also dominance and interaction effects. This way, fine mapping is performed (Kooke et al., 2012).

After the mapping population has been developed, sampling the population for genotyping is another essential step in QTL analyses. Many types of genetic markers are used, with the most common types being: single nucleotide polymorphisms (SNPs), simple sequence repeats (SSRs, or microsatellites), restriction fragment length polymorphisms (RFLPs), and transposable element positions (Miles and Wayne, 2008). Such markers do not affect the phenotype of the trait of interest. Three major types of genetic markers are present: (1)

morphological (visible) markers which are phenotypic characters themselves; (2) biochemical markers (allelic variants of enzymes) and (3) DNA (molecular) markers, which reveal sites of variation in DNA. DNA (molecular) markers are among each other divided into three major groups, largely based on their detection method: (1) hybridization-based; (2) polymerase chain reaction (PCR)-based and (3) DNA sequence-based (Collard et al., 2005). The ideal DNA markers usually have: high level of polymorphism, even distribution across the genome, co-dominant, clear distinct allelic features, single copy and no pleiotropic effect, low cost, easily detected, easily duplicated, available, genome specific and they do not harm phenotype in any way (Jiang, 2013). QTLs can be traced through their genetic linkage to a visible marker loci. Linkage mapping is tracing the linkage of a trait with a marker. If a QTL is linked to a marker locus, then individuals with different marker locus will have different mean value of the quantitative trait of interest (Mackay, 2009). Basically, genetic markers are an actual genetic difference between individuals or species in general. Since they are closely located to genes, they act as 'signs' or 'flags', more than the target genes themselves. Single nucleotide polymorphism (SNP) is one base pair position in a DNA sequence at which two (or more) individuals differ, which means that they have alternative alleles at the specific spot. The least frequent allele should have an abundance of at least 1% or greater. SNPs are one of the most popular molecular marker choices due to their abundance in genome. This has made the very useful for creating high-density genetic map which cannot be achieved with other genetic markers. High quality genotyping has a great potential with SNPs (Jehan and Lakhanpaul, 2006). Simple sequence repeat (SSR) markers or the microsatellites are widely used molecular marker types since they are PCR-based, genetically co-dominant, hyper-variable, highly polymorphic, robust, reproducible and amenable to automation (Synrem et al., 2017). SSRs are simple repeated motifs that contain 1 to 6 base pairs, and they can be found in both coding and non-coding regions. Inheritance mode as codominant markers in a Mendelian fashion is their primary advantage. Also, they have been popular markers thanks to their high polymorphism rates, high abundance and a broad distribution throughout the genome. Disadvantage is that they have relatively high development costs and technical challenges during the construction of species-specific primers (Miah et al., 2013). Restriction fragment length polymorphism

molecular (RFLP) marker is a piece of DNA extracted from the larger molecule by the restriction endonuclease enzyme. The polymorphism is determined by the number and varying lengths of these DNA fragments (Williams, 1989). Most of the RFLP markers are codominant and locus specific. RFLP genotyping is highly reproducible, and with the simple methodology that does not require any special equipment, RFLP is one of the preferred molecular marker choices. However, radioactive autography involved in RFLP genotyping has limited its sharing between laboratories, which led to decline in its use in the last decade. One of the downsides is also that RFLP requires large amounts of high-quality DNA and has low genotyping throughput. Amplified fragment polymorphism (AFLP) markers are PCR-based markers. Simply put, they are like RFLPs visualized by selective PCR amplification of DNA restriction fragments. Technically, AFLP is based on combining polymorphism at restriction sites and hybridization of random primers. AFLP is a very reliable, robust, good in case of small variations in PCR amplification parameters and it can produce a high marker density (Jiang, 2013).

A linkage map is a map that shows the frequencies of recombination that occurs between markers on homologous chromosomes. The distance between the two markers is proportional to the recombination frequency between those two markers. The distance between the two markers will be greater if the recombination frequency between them is greater. Also, smaller the recombination frequency is smaller the distance between two genetic markers. Shortly, linkage map represents recombination events and frequencies, rather than an actual physical map. In order to construct a genetic map, mapping population is needed whose parents exhibit both trait and marker polymorphism (Cone and Coe, 2009). Linkage maps have been used to identify chromosomal regions that contain genes controlling simple traits, as well as quantitative traits using QTL analysis. The process in which we construct linkage maps and conduct a QTL analysis in order to identify genomic regions associated with desired traits, is also known as QTL mapping (Collard et al., 2005). Genetic maps consist of markers positioned along a chromosome. It is a common practice to measure their relative locations in the map units known as centiMorgans. During meiosis, crossing over event occurs involving two strands of different origins (non-sister pairs). The average number of crossovers between a pair of markers that occurs during meiosis on one

chromatid is the genetic map distance in Morgans. Since crossovers are not visible, a genetic map function is used to show genetic distance from the observable recombination fraction. In 1919, scientist called Haldane wrote a paper that defined map functions by assuming no crossingover interference. He introduced an equation that allowed the construction of a variety of map functions (Zhao and Speed, 1995). On the other hand, scientist named Kosambi was pointing out the additivity problem. He has found out that interference had caused high inaccuracy in estimations. Kosambi's mapping function adjusted the map distance based on interference which changes the proportion of double crossovers. The new mapping function included interference and it explained that the crossover interference depends on the size of the chromosome segment, and that interference increases when the segment size decreases (Vinod, 2011).

After the immense work of creating the mapping population, genotyping and phenotyping, all acquired data should be statistically analysed in order to point out the putative QTLs, their effects and interactions. This is done through several statistical tests and methods.

QTL analysis is performed by testing the effect of each marker on the linkage map of the desired trait. This can be performed by an advanced analysis of variance (ANOVA). It shows us if the additional variance observed after a change in QTL alleles have arisen just by chance or due to the change in QTL. In standard ANOVA, the residual variance within the QTL genotype classes is compared with the mean values of the classes, and later they are statistically tested with a t-test or F-test. Sometimes called single marker ANOVA, this test is performed for each segregating marker at the time, and the results are compared to the marker position (Alonso-Blanco et al., 2006). There are other methods, one of which is an Interval mapping (IM). Interval mapping is a statistical approach developed to determine which of the QTLs on the map can be in a previously prepared map of the marker locus. It uses maximum likelihood method. Maximum likelihood method requires the use of statistical models with more intensive probability calculations (Şahin and Kavuncu, 2019). In standard IM, each locus is considered one at a time and the logarithm of the odds ratio (LOD) is calculated considering that the locus is a true QTL. LOD is related to the Pearson

correlation coefficient between the phenotype and the genotype for each individual in the experimental cross (Alam et al., 2019). Compared to a single-marker analysis, IM allows us to test QTL effects at every map position, and not just at the marker positions (Alonso-Blanco et al., 2006). Since Maximum likelihood methods can be complex and very computationally slow, Haley-Knott regression has been also in use. In this method, QTL mapping is based on multiple regression which can be used with any general statistical package (Haley and Knott, 1992). Another method has been developed for more precision mapping. It is known as Composite Interval Mapping (CIM) and this method is based on a combination of interval mapping with a multiple regression analysis. This method can locate multiple QTLs more accurately and efficiently (Zeng, 1993). Multiple Interval Mapping (MIM) is a method that uses multiple marker intervals simultaneously to fit multiple presumed QTLs directly into the model for mapping QTL. This method, unlike others, gives us a possibility to find epistasis between QTL, genotypic values of individuals, and heritability of quantitative traits (Kao et al., 1999). Bayesian method for detecting QTLs is based on multiple regression models with trait values regressed on marker genotypes. It uses a Bayesian information criterion to estimate the posterior probability of models with various subsets of markers as variables (Ball, 2001). In order to be sure if found QTL is truly reliable, we should observe if the LOD score is above the threshold. The method of checking the presence of linkage between two loci (markers) created on its basis and widely used in genetic investigations was called the LOD score (logarithm of the odds ratio score) (Kocherina et al., 2011). The score implicates the strength of evidence for the presence of a QTL. Larger the LOD score - there is a greater evidence for QTL presence. LOD scores must be closer to 3 before they will generally be deemed interesting. A LOD score of 3 shows that there is 1,000 times more likely to get the observed data, if there is a QTL at the specified position than if there are no QTLs. Many statisticians have expended the estimation of the LOD threshold to a P value of 5% (Karl W. Broman, 2001).

2.2 QTL research in the Brassica Family

Scientists have already started implementing QTL analyses technology and methods on *Brassica* family as well as cabbage species and its different cultivars.

Wang et. al. (2012), have worked on construction of high-density linkage map in cabbage. They have used two diverse inbred lines of cabbage in order to create a doubled haploid mapping population. SSR and SNP markers were used for map construction. In total, 8 497 DNA markers were screened in the parental lines resulting in 15% of them polymorphic. 602 SSRs and 625 SNPs were assigned to nine linkage groups that correspond to the number of cabbage chromosomes. The high-density genetic map was constructed with the average genetic interval of 0.98 cM and the average physical intervals of 503.3 Kbp per marker. This was the one of the most saturated linkage maps for *B. oleracea* in recent research. The main purpose of was to anchor scaffolds onto the pseudochromosomes. Approximately 83% of *B. oleracea* genome has been included into this genetic map. Ideally, the average distance between the two neighbouring markers should be short and evenly distributed, however here it is not a case. This is because skewness commonly appears in DH populations, since there was different reaction to the chemical treatment of the microspores. Authors suggested that mapping with different mapping population types as well as different molecular marker types is needed (Wang et al., 2012).

Another scientific work has dealt with cabbage genetic maps. Lv et al. (2016) have performed detailed QTL mapping for 24 agronomic traits of cabbage. In order to improve marker assisted selection breeding (MAS), they have used a doubled haploid mapping population. Trials were conducted in three seasons on 196 DH lines, F1 progeny and their parents. Plant, leaf and head type related traits were carefully measured according to the previously determined standards for cabbage at both rosette and harvest stage. MapQTL4.0 program was used for the QTL analysis, by using interval mapping and multiple-QTL model mapping. As a result, 144 QTLs with LOD threshold higher than 3, were detected. 95 of them have contributed more than 10% to the variation (Figure 2.1). Since most of the QTL analyses in cabbage have been concentrated on the disease resistance, this article has given valuable data on other traits that contribute to the yield and are important to both growers as well as cabbage breeders (Lv et al., 2016).

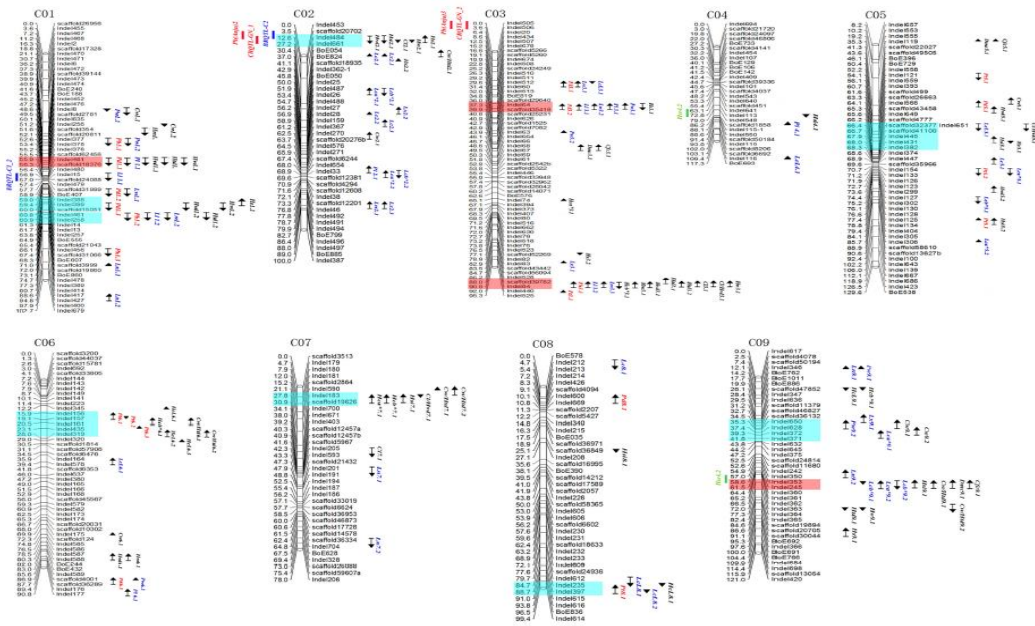


Figure 2.1. QTL mapping for cabbage agronomic traits in genetic linkage maps (Lv et al., 2016).

Since heading is a very important agronomic trait that determines yield and quality in cabbage, QTL analyses were conducted in this area too. Zhang et al. (2016) have conducted a study in which they have mapped QTLs for heading traits. They used 130 lines of DH population derived from one oblate head inbred parent and one round head inbred parent. Experiment was conducted for two years. For phenotypic evaluation, head vertical diameter, head transverse diameter and head shape index were head shape related traits measured according to the standards. 2170 SSR and 149 pairs of InDel primers were used to search for polymorphisms between the two parents. Genetic distances between markers were calculated according to the Kosambi function, while the genetic map was constructed using JoinMap 4.0. 48 InDel and 149 SSR markers were used in genetic map construction with a total length of 866.2 cM and an average interval length of 4.40 cM. Inclusive composite interval mapping (ICIM) using QTL IciMapping v3.0 was used to detect QTLs. Based on LOD thresholds (calculated for a type I error rate of 0.05), interval mapping at 1-cM intervals was used to scan for QTLs. Results showed fourteen QTLs associated with head shape which were distributed across seven chromosomes. Two major QTLs, on chromosomes 3 and 8 were detected in both years, and they explain 19.16–24.56% and 11.24–

21.55% of the phenotypic variation, respectively. This was the first research to find QTLs of the traits of head transverse diameter and head shape index (Zhang et al., 2016).

One of the important problems regarding the quality and yield of cabbage is head splitting. Sometimes, during later stages of growth, cabbage heads tend to crack. This seriously affects the appearance, yield and mechanical harvest ability of the cabbage. Resistance to head splitting is believed to be a complex trait, controlled by many genes. The research of Su et al. (2015) has aimed at identifying major QTLs responsible for head cracking in order to help breeders by selecting the right lines for their future work in creating more resistant to head cracking cultivars. DH population was derived from F1 generation of a female susceptible and male resistant to head cracking parental inbred lines. For phenotyping purposes, splitting was assessed on a 0 to 5 scale. The numerical mark was given according to the number of split layers and the ratio of the split size to the entire surface area. SSR and InDel markers were used for linkage map construction, using JoinMap 4.0 software. Multiple-QTL modelling (MQM) with MapQTL 4.0 software and inclusive composite interval mapping (ICIM) with QTL IciMapping v3.0 software, were used to detect QTLs. Results yielded in nine QTLs on chromosomes no. 3, 4, 7 and 9. They have explained individually 5.50–16.93% of the observed phenotypic variation. Also, they have found significant negative correlation between the head splitting resistance and the head vertical diameter, head transverse diameter and head weight. Even though there are head splitting resistant varieties, this trait is heavily dependent on environmental factors, so having DNA markers for marker assisted selection could have a great importance for breeding (Su et al., 2015).

Great importance for breeding programs is also in elite lines. Those lines are associated with excellent economic traits. This is the reason why they are heavily used in plant breeding, in order to generate many new excellent varieties. Also, they are a good material for studying the genetic effects of the loci associated with excellent economically important traits. Lv et al. (2017) has conducted a research in which they did a genome wide scan to reveal significant loci connected to important agronomical traits in elite cabbage line ‘01-20’. This line is known for

its green outer leaves, a more compact and upright plant-type, rounder head, shorter core length, and better taste. ‘01-20’ and its five sister lines (‘01-07-258’, ‘01-07-251’, ‘01-1-4’, ‘01-88’, ‘01-16-5’) were used in the experiment. 25 agronomical head, leaf and core related traits were phenotyped and assessed by standards prescribed in ‘Descriptors and data standards for cabbage’. Genotyping was performed with 406 pairs of InDel and SSR markers for all cabbage materials. Results included a discovery of eight unique loci for ‘01-20’ and ‘01-07-258’. Five of these loci showed an association with important agronomic traits, based on QTL association results. This could explain why this elite line is so successful in breeding programs. However, authors stated that the underlying danger of loss of the genetic diversity can be due to overuse of the same elite lines (Lv et al., 2017).

Since cabbage hybrids exhibit high heterosis vigour, they are commonly used in cabbage production. Even though, self-incompatible lines and male-sterile lines are two common tools for hybrid cabbage seed production, they are labour intensive, and the purity of hybrid seeds never reaches 100%. Self-incompatibility is the mechanism which prevents self-fertilization in plants by recognizing and rejecting the plant’s own pollen is called. Although self-incompatibility is common in cabbage, some studies have shown that S-locus genetic variation can be responsible for self-compatibility. Producing self-compatible lines could reduce costs during the parental line reproduction process and avoid crossing incompatibility during the hybridization. In order to improve cabbage breeding, Xiao et al. (2019) have conducted a study with the aim of finding QTLs associated with self-compatibility. In order to understand the genetic basis of self-compatibility, whole-genome mapping of the QTLs was performed on an F₂ population derived from a cross between parental line “87-534” and parental line “96-100”. Crossing incompatibility (CI) value, alongside with fluorescence microscopy were used to assess the self-compatibility phenotype. To identify the polymorphic markers, InDel primers were used for whole-genome scan of the parental lines, then individual F₂ plants were screened with the polymorphic markers. A linkage map was constructed using the Join Map 4.0 program with a LOD score of 4.0. To convert the recombinant value to genetic distance, Kosambi function was used. Eight QTLs with high contribution rates (CRs) for three QTLs

were detected. These three QTLs' linkage markers were used to develop self-compatibility marker combinations. The introgression of self-compatibility from 87–534 to 96–100 was successful. The result of the research were self-compatibility-specific marker combinations and 36 genome-wide markers for background selections. A high selection efficiency was achieved in the transferring process could greatly improve the cabbage breeding efficiency (Xiao et al., 2019).

Xanthomonas campestris pv. *campestris* is a gram-negative bacterium that causes one of the most destructive diseases for *B. oleracea* crops, the black rot disease. Disease symptoms are chlorosis on the edges of the leaves, necrosis and darkening of the leaf veins and of the vascular tissue of the stem. Later, the disease will result in wilting and necrosis throughout the whole plant. *Xcc.* is the reason behind great economic losses, reduced performance and the bad quality of the *Brassica* plant crops. By far, there is no effective method to control *Xcc.* Therefore, Iglesias-Bernabé et al. (2019) have conducted a research with the aim to analyze the quantitative resistance to this disease in leaves of *B.oleracea* by QTL analysis. 137 doubled haploid lines (BolTBDH) were used as mapping population for the experiment alongside with *Xcc.* Race 1 type strain HRI3811 that was used for the resistance test. Resistance was tested with two inoculations and five traits were recorded in both. These traits are: initial index (INDEX0), final index (INDEXF), diseased leaf area (DISAREA), percentage of diseased leaf area (%DISAREA) with respect to total leaf area (TOTAREA), and percentage of points showing symptoms (%DISPOINTS). These traits are related to initial stages of the invasion, success of infection and spread of the pathogen. Analyses of variance were performed for disease traits. Pearson's correlation coefficients were computed between the resistant traits. Linkage map was generated with 279 markers (SSRs and RFLPs). PLABMQTL22 software was used to detect single-trait QTLs through a composite interval mapping method (CIM). In order to prove the significance of the putative QTL, LOD threshold was chosen for each trait with permutation test (N=1000) and a critical alpha value of 10%. Genstat version 19.1.0.2139 software was used to detect multi-trait QTL. A genome wide significance of $p < 0.0524$ was used as threshold for QTL detection. Results have shown that, although some of the individuals were more resistant than parents,

almost the entire population showed symptoms at all points of inoculation. Four resistance trait related single QTLs were detected, from which one represents the novel variation. Multi-trait QTL analyses has shown that spreading of *Xcc* is related to the size of the leaf. It was found that plants with P1 alleles have a reduced lesion size as a resistance mechanism, while genotypes with P2 alleles are keeping more area of the leaf with photosynthetic activity as a coping mechanism. These findings could improve MAS resistance breeding of the *Brassica* crops (Iglesias-Bernabé et al., 2019).

Due to the importance of *X.campestris* pv. *campestris* (Black rot disease) for cabbage production, the identification of resistance QTLs has great importance. Lee et al. in their research in 2015, have performed a whole-genome resequencing of two cabbage parental lines and genome-wide SNP identification. In their research, previously published *B. oleracea* genome sequences have been used as a reference. Large numbers of reliable SNPs were obtained and utilized them for development of DNA markers, which were able to improve the genetic map of cabbage. New linkage map spanned across 1,467.3 cM (135.4 cM more than the previous one). Three independent inoculation trials were performed in a period of three years. F2 population was used and even though the same inoculation test conditions were conducted, the disease symptoms were not the same and they have worsened over the years. JoinMap version 4.1 software was used for linkage analysis and map construction. Recombination frequencies were converted into genetic distances with the Kosambi mapping function. Disease index was used as phenotypic data. QGene program was used to perform composite interval mapping (CIM) analysis in order to detect QTLs. LOD score threshold was estimated with permutation test (N=1000) at 5% significance. The QTL analysis has resulted in four QTL regions that could contribute additively to resistance. The BRQTL-C1_2 region has been repeatedly detected in all of the three independent inoculation tests. It had the highest LOD values. Also, it has shown to be a reason behind the highest variation percentage in all tests. The identified QTL regions have 21 candidate resistance genes (Lee et al., 2015).

Alongside with cabbage, other members of the *Brassica* family were also a subject of extensive QTL analyses projects. Chinese cabbage (*Brassica rapa* L.

ssp. pekinensis) is one such example and it is one of the most important vegetables in China. Since it is one of the main sources of people's mineral nutrients, Wu et al. (2008.) have decided to map QTLs for mineral accumulation in order to aid in work of biofortification of this vegetable. Their goal was to analyse Chinese cabbage plants for the concentration of 11 elements in leaves (Fe, Zn, Mn, Cu, Na, Ca, K, P, Mg, Sr and one toxic element Al) and then detect QTLs for mineral accumulation and for shoot dry biomass yield under normal, deficient and excessive Zn conditions. 183 double haploid lines were used as a mapping population. Linkage map was constructed with 203 AFLPs, 58 SSRs, 22 SRAPs and four ESTPs markers. JoinMap version 3.0 software was used for linkage map construction. Recombination frequencies were converted into genetic distances with the Kosambi mapping function. The parents and 142 DH lines grown in an open field and their leaf mineral concentration was analysed. Mineral concentration was determined by inductively coupled plasma-atomic emission spectrometry. For the inspection of Zn deficiency and toxicity effect on nutritional stress in shoot dry biomass, the parental lines and 140 randomly selected double haploid lines were hydroponically grown in a greenhouse without climate control. For the purpose of statistical analysis, mineral concentration data were log₁₀-transformed to improve the distribution normality and be used in QTL analyses. MAPQTL version 5.0 software was used to identify and locate QTL on the linkage map. Interval mapping (IM) and multiple-QTL model (MQM) mapping methods were applied. Analysis of variance (ANOVA) at a significance level of $p \leq 0.05$ was used to the interaction between the QTLs of the same trait. The results have yielded in 11.1–17.1% of the Na, Mg, P, Al, Fe, Mn, Zn and Sr concentration variance explained by detection of ten QTLs. Shoot dry biomass under normal, deficient and excessive Zn had one common QTL. These results could help in breeding for biofortification (Wu et al., 2008).

In case of another *Brassica* crop, broccoli, abiotic type of stress, heat stress was investigated. For USA market, broccoli is mainly produced in California, state with high temperatures and drought which is not beneficial weather for the broccoli, traditionally cool season crop that grows in 15 to 23°C temperature ranges. Therefore, Branham et al. (2016) have searched for QTLs that could increase broccoli's heat tolerance. 155 doubled haploid individuals were used as a

mapping population. Scale from 1 to 9 (with 9 being the highest quality and 1 as unrecognizable) was used to assess heat tolerance at peak head maturity in three heads per plot. SNP markers were used to develop a linkage map. Linkage map was constructed with R/qtl program. LOD threshold of 25 was used. Markers were set in order within linkage groups by the ripple function with a window size of 7. Recombination frequencies were converted into genetic distances with the Kosambi mapping function. QTL detection was performed with R/qtl program by Standard interval mapping with Haley–Knott regression. Multiple QTL mapping (MQM) was performed and model was chosen based on LOD threshold determined with permutation (N=1000). Five significant QTLs and one epistatic interaction were identified, accounting for 62% of the variation in heat tolerance. Heat tolerance alleles from the QTL on linkage groups 2, 3, 5 and 7 came from the heat-tolerant parent (USVL138), while interestingly, the positive effect from the QTL on linkage group 9 came from the heat-sensitive parent (USVL108) (Branham et al., 2016).

Cauliflower (*B.oleracea* var. *botrytis*) has also been a part of *Brassica* QTL research topics. Since cauliflower harvest time is delayed by high temperatures, it would be useful to explain the genetic regulation of floral transitions in order to have more precise harvest scheduling and ensure stable market supply. Matschegewski et al. (2015) have dealt with this question by identifying QTLs responsible for cauliflower curding under two different temperature regimes. A diverse set of 111 *B. oleracea* var. *botrytis* commercial parent lines were used for phenotyping. *B. oleracea* var. *botrytis* DNA was extracted and then hybridized to an Illumina Infinium iSelect according to protocols which resulted in 14 385 polymorphic SNP markers used for genotyping. Kruskal-Wallis test and ANOVA were used for statistical analyses of phenotypic data. 18 QTLs were found on chromosomes O1, O2, O3, O4, O6, O8 and O9 for curding time under different temperatures. These data could be used in marker assisted selection breeding in order to produce more thermo stable cauliflower cultivars that could bring more stability to the market supply (Matschegewski et al., 2015).

When it comes to general breeding goals of cabbage cultivars, traditionally, high and reliable yield is still number one priority. However, in order to satisfy the

consumer market and beat the competition, greater attention was paid to the quality of cabbage. Crisp compact heads, core length until the half of the head height, good flavour and high nutrient value are just some of the quality parameters that should be taken into the consideration. With world becoming a global village and disease spreading faster and easier than ever, resistance to diseases such as Black rot (*X.campestris* pv. *campestris*), fusarium wilt (*Fusarium oxysporum* fs. *conglutinans*), downy mildew (*Peronospora parasitica*), powdery mildew (*Erysiphe polygoni* D.C.), bottom rot (*Rhizoctonia solani* Kuhn) etc. has become a common imperative in breeding goals and schemes. Some problems like early bolting can cause heavy economic losses especially in the year with sudden frosts in spring season, so it is important to breed early bolting resistant varieties. If we wish to achieve cabbage varieties with early maturity characteristics, cold and heat tolerance is required. In case cabbage is stored in the winter, compact head and storage are needed characteristics. Crop uniformity and all year-round supply would greatly help farmers achieve easier and more profitable production. Colour and shape are another important characteristics; however, they are highly dependent on consumer preferences based on their region and habits (Fang et al., 2005).

As it is visible from scientific papers overview stated above, many of these subjects were investigated through QTL analyses in cabbage as well as in its close relatives, other members of the *Brassica* family. Plant, head, leaf type related characteristics as well as other yield and agronomic related traits were analysed (Lv et al., 2016; Su et al., 2015; Zhang et al., 2016). Disease resistance, especially resistance to the Black rot (*X.campestris* pv. *campestris*) has also been a subject of the QTL mapping research (Iglesias-Bernabé et al., 2019; Lee et al., 2015). Meanwhile abiotic stress tolerance QTLs were also detected for heat stress in broccoli (Branham et al., 2016) and for curding under two distinct temperatures in cauliflower (Matschegewski et al., 2015). As an important subject of research in latest years, biofortification was also explored through QTL mapping in Chinese cabbage type with the aim to increase micro and macro nutrient concentration (Wu et al., 2008). These results suggest that QTL analyses is a powerful tool in marker assisted selection and breeding, and could aid cabbage breeders, as well as all plant breeders in number of problems and breeding goals.

3. MATERIALS AND METHODS

3.1 Parent Materials and Field Experiments

The field experiment was performed at the vegetable experimental station of Syngenta, in Ócsa, Hungary, in period between February and June 2020. Hungary is a central European landlocked country characterised by temperate climate with warm summers and cold winters and heavier rainfall during spring and autumn. The present-day location of the experimental station was a swamp in the past. Thus, the soil is rich in organic matter and very suitable for agriculture. Syngenta Ócsa experimental station has 23 ha of open fields, 6000 m² of heated tunnels, 1200 m² of unheated tunnels and 600 m² of greenhouse, 2 seed storage rooms for short and long storing (with temperature of 11°C and 4°C, respectively).

Two parental lines referred as P1 and P2 were used to develop the mapping population come from Syngenta white cabbage germplasm collection. These are early maturing lines, which can be grown in approximately 50 days.

Parent 1 (“P1”) is a Doubled Haploid line that exhibits strong vegetative growth with deep green outer leaf plant colour and darker green head colour, poor head density and long core length (Figure 3.1). Parent 2 (“P2”) is a Doubled Haploid line that exhibits good generative growth with bright green outer leaf plant colour and light green head colour, good head density and short core length (Figure 3.2).



Figure 3.1. Parent 1 typical plant; (left) whole plant view and (right) head cross - section view.



Figure 3.2. Parent 2 typical plant; (left) whole plant view and (right) head cross - section view.

“P1” was crossed with the “P2” in order to generate F1 population (Figure 3.3) from which F2 mapping population was obtained. “Pandion” was used as control check variety (Figure 3.4).



Figure 3.3. F1 population obtained from "P1" and "P2" cross; (left) whole plant view and (right) head cross - section view.



Figure 3.4. “Pandion” control check variety; (left) whole plant view and (right) head cross - section view.

F2 population plant individuals were used for the QTL analyses. 1428 seeds were sown on 16th January 2020 in the greenhouse. 42 seeds of “P1”, 42 seeds of “P2” and 1344 seeds of “F2” population were sown. Altogether, 955 plants were transplanted to the field. 25 plants of “P1”, 20 plants of “P2” and 910 plants of F2 population were transplanted to the field on 18th March 2020. Plants were receiving approximately 15mm of irrigation on the weekly basis. Fertilization was applied regularly according to the general local practices. Due to the heavy Cabbage Root Fly (*Delia radicum*) larvae infestation, plant protection treatment has been applied four times, on 24th April, 4th May, 7th May and 11th May 2020. All materials were evaluated in the period from the 18th May until the 1st June 2020.

3.2 Data Collection

Altogether 13 traits were measured. These traits are: Stem length, Outer leaf length, Outer leaf width, Leaf erectness, Head weight, Colour, Head length, Head width, Core length, Head shape, Head density, Anthocyanin discoloration, and Vigour of the plant.

It is important to note that due to the Syngenta Company’s Privacy Policy, I will not be able to show results of Core length QTL analyses even though I have measured it.

Stem length was measured as distance of the top of the stem and ground, in centimetres (Figure 3.5). Leaf erectness was measured according to the angle outer leaves have, on a scale from 1 to 9. 1 was the worst mark representing the outer leaf laying completely horizontally, at the angle of 0, while 9 was the best mark representing the leaf standing vertically, at the angle of 90° (Figure 3.5). Outer leaf length and width were measured as length and width of the biggest outer plant leaf, in centimetres (Figure 3.6).



Figure 3.5. (left) Stem length measurement, red arrow shows the stem portion measured. (right) Leaf erectness measurement, red arrows show how 1 – 9 marks were given according to the leaf angle.



Figure 3.6. (left) Outer Leaf length, represented by red arrow, and (right) Outer Leaf width, represented by red arrow.

Vigour was measured on the scale from 1 to 9, with 1 being the worst mark, representing plants with small stunted growth, and 9 being the best mark representing strong vigorous plants (Figure 3.7). Anthocyanin discoloration was measured on a scale from 1 to 9, with 9 being the best mark and meaning absolutely no anthocyanin discoloration and 1 being the worst mark, meaning high leaf anthocyanin discoloration coverage (Figure 3.8).

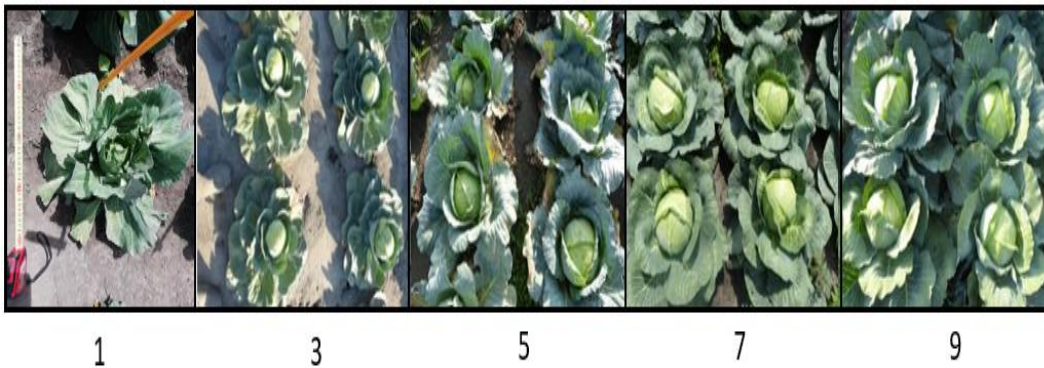


Figure 3.7. Vigour measurement scale with 1 - 9 marks.



Figure 3.8. Anthocyanin discoloration scale from 1 to 9; red circles point out the size of the anthocyanin damage.

Cabbage head weight was measured on a scale, in grams (Figure 3.9). Colour was measured on a scale from 1 to 9 with 1 being very light green colour and 9 being very dark green colour. Head length was measured as the maximum vertical length of a cut – open head from the bottom to the top of the head, in centimetres (Figure 3.10). Head width was measured as the maximum horizontal length of a cut – open head from at the widest point of the head, in centimetres (Figure 3.10). Core length was measured as the maximum vertical length from the

bottom of the head until the top of the core, in centimetres (Figure 3.10). Head shape was calculated as Head height to Head width ratio (HH/HW). If the HH/HW ratio was less than 0.8, head was considered flat, if the HH/HW ratio was more than 1.1, head was considered elongated, and if the HH/HW ratio was between 0.8 and 1.1, head was considered round (Figure 3.11). Head density was measured on the scale from 1 to 9 with 1 meaning that head has very loose leaves inside a cut – open head, while 9 means that head has very dense leaves inside a cut – open head (Figure 3.12).



Figure 3.9. Head weight measurement in field.



Figure 3.10. (left) Head length, represented by red arrow; (middle) Head width, represented by red arrow; (right) Core length, represented by red arrow.



Figure 3.11. Head shape represented by three main types: (left) flat, (middle) round and (right) elongated.

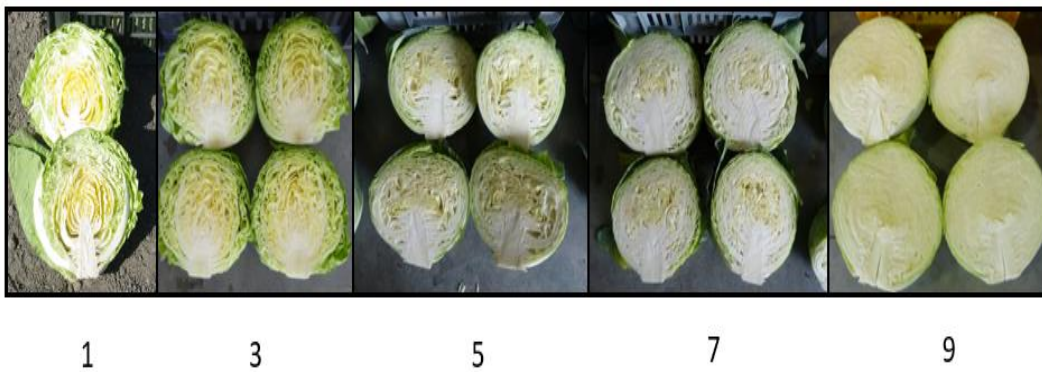


Figure 3.12. Head density, measurement scale with 1 - 9 marks.

3.3 QTL Analyses

15 000 SNP markers were used for fingerprinting, out of which 2400 were polymorphic and used to build Linkage map. QTL analyses was performed in R x64 3.6.2 program, with R/qtl package (Broman et al., 2003). Due to the R/qtl limitations, only 369 polymorphic markers were used for QTL analyses. The “calc.genoprob” R/qtl function was used to calculate genotype probabilities under error probability of 0.01 and a map function “Kosambi”. The “scanone” R/qtl function was used to perform an initial round of interval mapping, with estimated genotyping errors of 0.001 using Haley Knott regression. The “cim” R/qtl function was used to perform Composite Interval Mapping. Multiple QTL analyses model was searched using an iterative process (“fitqtl”, “addqtl” and “refineqtl” R/qtl functions). Function ‘effectplot’ was used to plot the phenotype

averages for the genotype groups at desired major QTL marker positions. LOD thresholds were obtained using permutation test $N=1000$. 1.5 LOD confidence intervals were used. In order to produce linkage group maps with QTL intervals marked on the map, Linkage Map View package for R/qtl software was used (Ouellette, 2018). Putative QTLs were named in the following manner: trait name abbreviation (Table 3.1), chromosome number and a QTL code, respectively. For example: Stem length has five QTLs, and we wish to mention a third QTL which is on the 6th chromosome. We will note it as: StLen.6.3.

Table 3.1. List of abbreviations for cabbage agronomic traits

Trait name	Trait abbreviation
Stem Length	StLen
Outer leaf length	LfLen
Outer leaf width	LfWid
Leaf erectness	LfEre
Head weight	HdWei
Head length	HdLen
Head width	HdWid
Head shape (HH/HW ratio)	HdRat
Head density	HdDen
Colour	Col
Anthocyanin discoloration	Ant
Vigour of the plant	Vig

4. RESULTS

4.1 Stem Length

The LOD score threshold at 5% for the Stem length was 3.45 and for 1% was 4.10 (Table 4.1). After performing Composite Interval Mapping (CIM), four putative QTLs were detected on chromosomes 1, 2, 6, and 9 (Table 4.1), and they had LOD scores higher than 3.45 (Figure 4.1). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 2 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Stem length. The five putative QTLs were determined at the following positions: at 107.33 cM on chromosome 1, at 17.86 cM on chromosome 2, at 138.86 cM on chromosome 2, at 111.50 cM on chromosome 6 and at 84.06 cM on chromosome 9 (Table 4.2). Multiple QTL analyses model explains for the 32.37% of the phenotypical variation with StLen.2.2 being the major QTL which explains 19.05% of variation with LOD score of 48.1 (Table 4.2). Function ‘effectplot’ was used to plot the phenotype averages for the genotype groups at StLen.2.2 QTL marker position (Figure 4.2). R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.3).

Table 4.1. Stem Length: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		name in R	chromosome	position	LOD
5%	3.45	AXO5405	1	101.10	3.583
1%	4.10	c2.loc18	2	19.40	47.391
		AXO8232	3	166.50	2.804
		AXO12215	4	110.20	1.194
		c5.loc44	5	44.00	2.493
		c6.loc112	6	112.00	18.495
		c7.loc113.5	7	113.5	1.706
		c8.loc82	8	82	0.721
		AXO1358	9	84.1	5.427

Table 4.2. Stem length: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model, P value and a major QTL (in bold); R/qtl

QTL	Chromosome	position (cM)	LOD	Variation (%)	P value (F)
StLen.1.1	1	107.33	3.13	1.09	0.000813
StLen.2.2	2	17.86	48.02	19.05	< 2E-16
StLen.2.3	2	138.86	9.9	3.5	1.69E-10
StLen.6.4	6	111.50	19.32	7.01	< 2E-16
StLen.9.5	9	84.07	4.91	1.72	1.43E-05
TOTAL:			85.28	32.37	

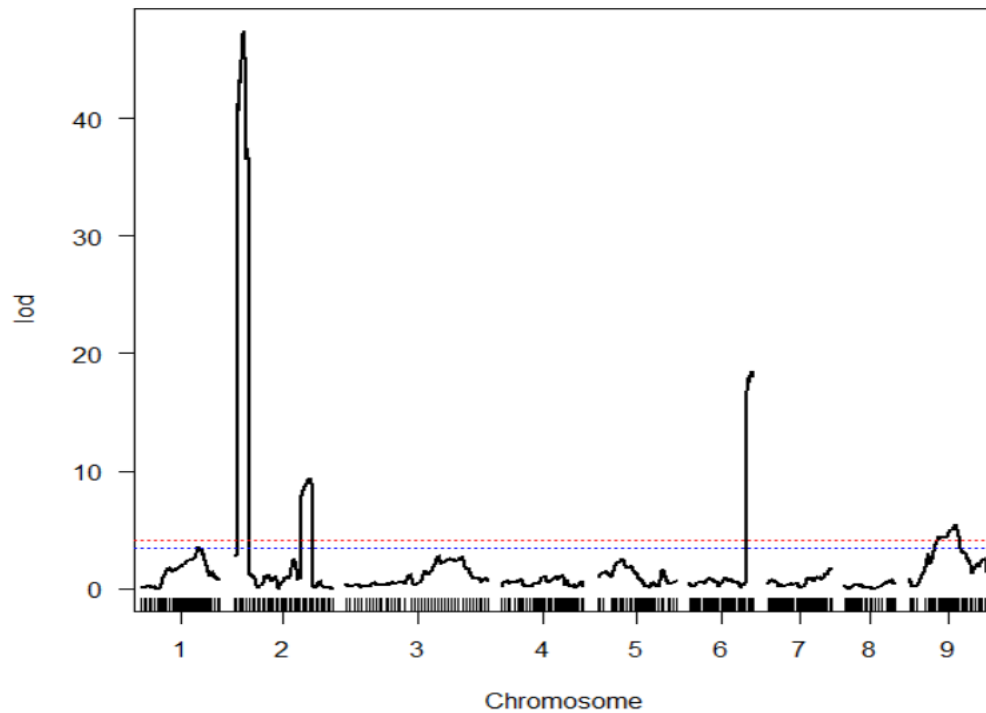


Figure 4.1. Stem length: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/qtl.

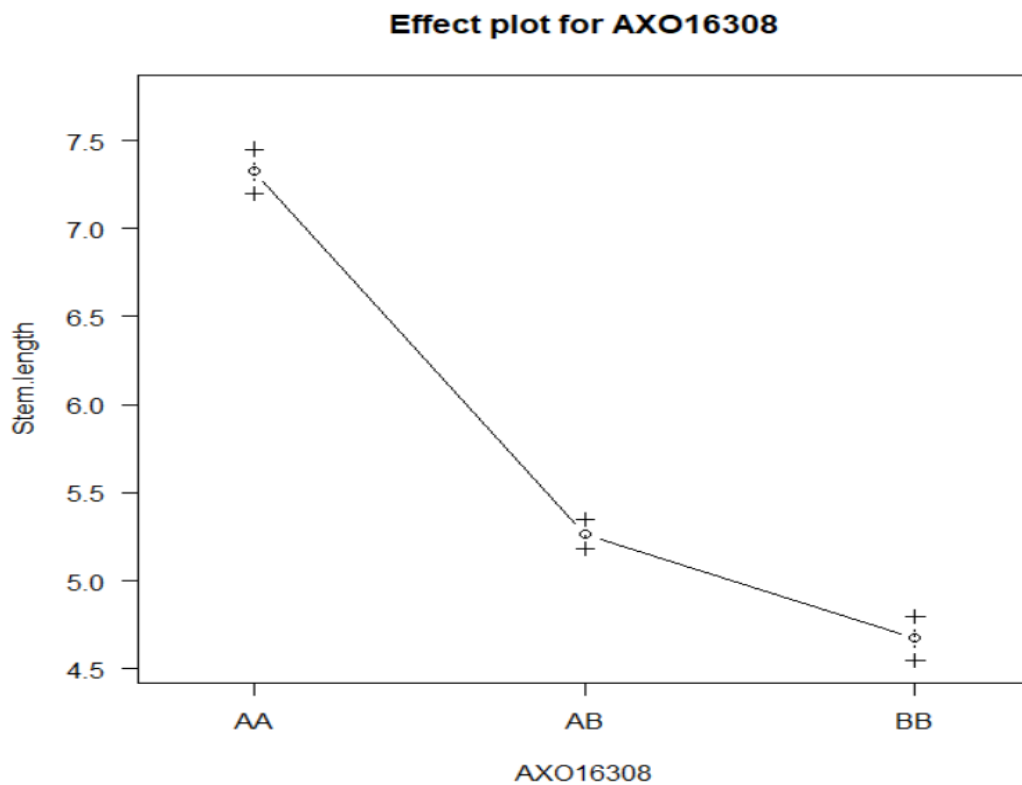


Figure 4.2. Stem length: Effect plot for the major QTL StLen.2.2.

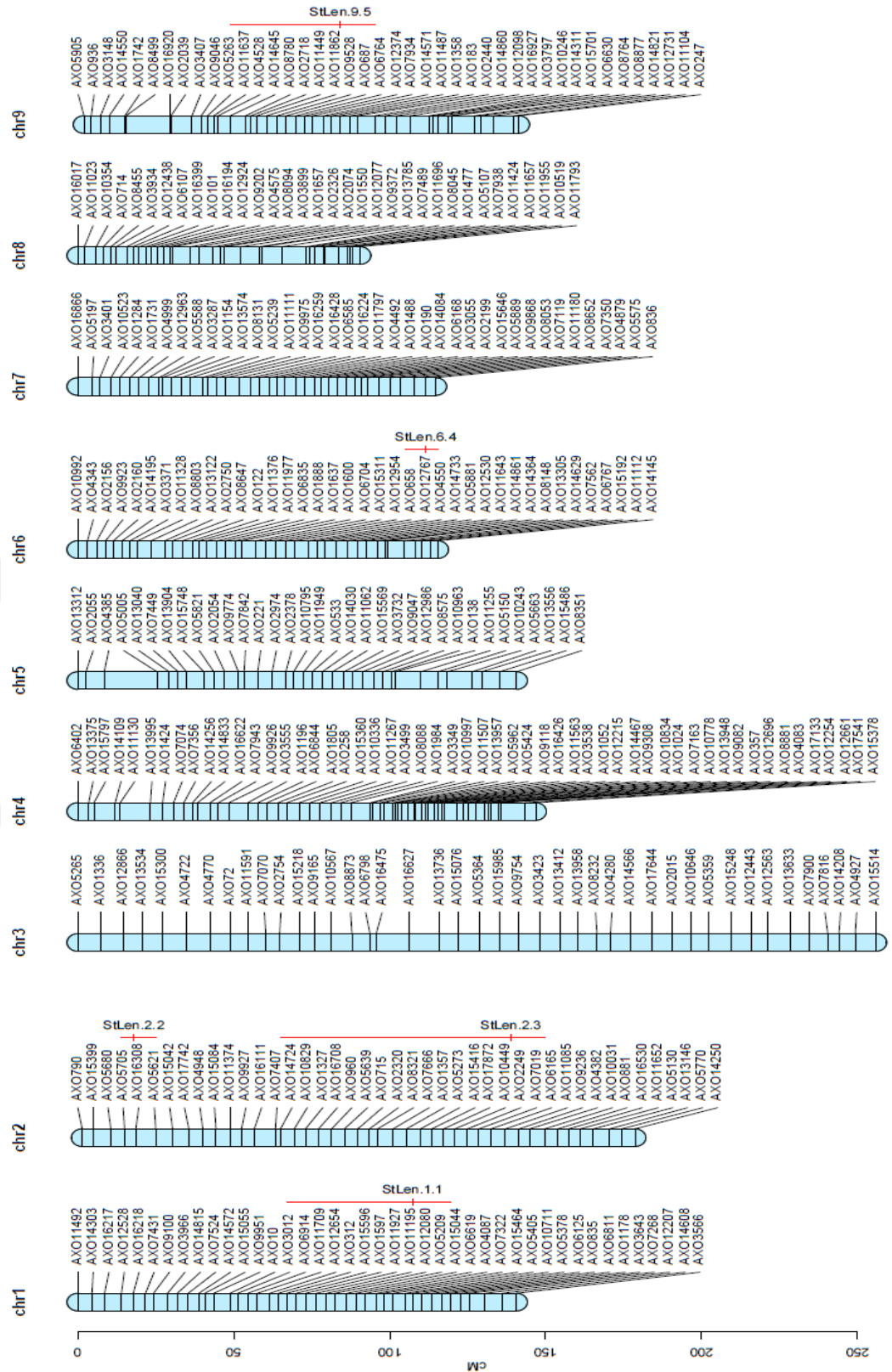


Figure 4.3. Cabbage Linkage map with marked QTLs for Stem Length.

4.2 Outer Leaf Length

The LOD score threshold at 5% for the Outer Leaf length was 3.67 and for 1% was 4.32 (Table 4.3). After performing Composite Interval Mapping (CIM), seven putative QTLs were detected on chromosomes 2, 3, 4, 5, 7, 8, and 9 (Table 4.3), and they had LOD scores higher than 3.67 (Figure 4.4). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. No additional QTL positions were found, and existing QTL positions were refined. There was no epistasis among the QTLs for the Outer Leaf length. Final result were seven putative QTLs at the following positions: at 130.36 cM on chromosome 2, at 145.50 cM on chromosome 3, at 33.50 cM on chromosome 4, at 55.00 cM on chromosome 5, at 25.95 cM on chromosome 7, at 5.88 cM on chromosome 8 and at 72.69 cM on chromosome 9 (Table 4.4). Multiple QTL analyses model explains for the 24.72% of the phenotypical variation (Table 4.4). There was no major QTLs. R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.5).

Table 4.3. Outer Leaf Length: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.67	AXO11492	1	0.00	1.49
1%	4.32	c2.loc132	2	133.36	6.75
		c3.loc122.5	3	122.5	8.41
		c4.loc33.5	4	33.5	4.19
		c5.loc123	5	123.00	4.23
		c6.loc90	6	90.00	1.91
		AXO5588	7	25.95	6.85
		AXO10354	8	5.88	8.53
		c9.loc72	9	73.97	9.03

Table 4.4. Outer Leaf length: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model and a P value; R/ctl

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
LfLen.2.1	2	130.36	9.35	4.12	6.58E-10
LfLen.3.2	3	145.50	10.30	4.55	8.02E-11
LfLen.4.3	4	33.50	4.78	2.08	2.03E-05
LfLen.5.4	5	55.00	4.30	1.87	6.05E-05
LfLen.7.5	7	25.95	8.75	3.75	2.57E-09
LfLen.8.6	8	5.88	8.62	3.80	3.48E-09
LfLen.9.7	9	72.69	10.29	4.55	7.87E-11
TOTAL			56.39	24.72	

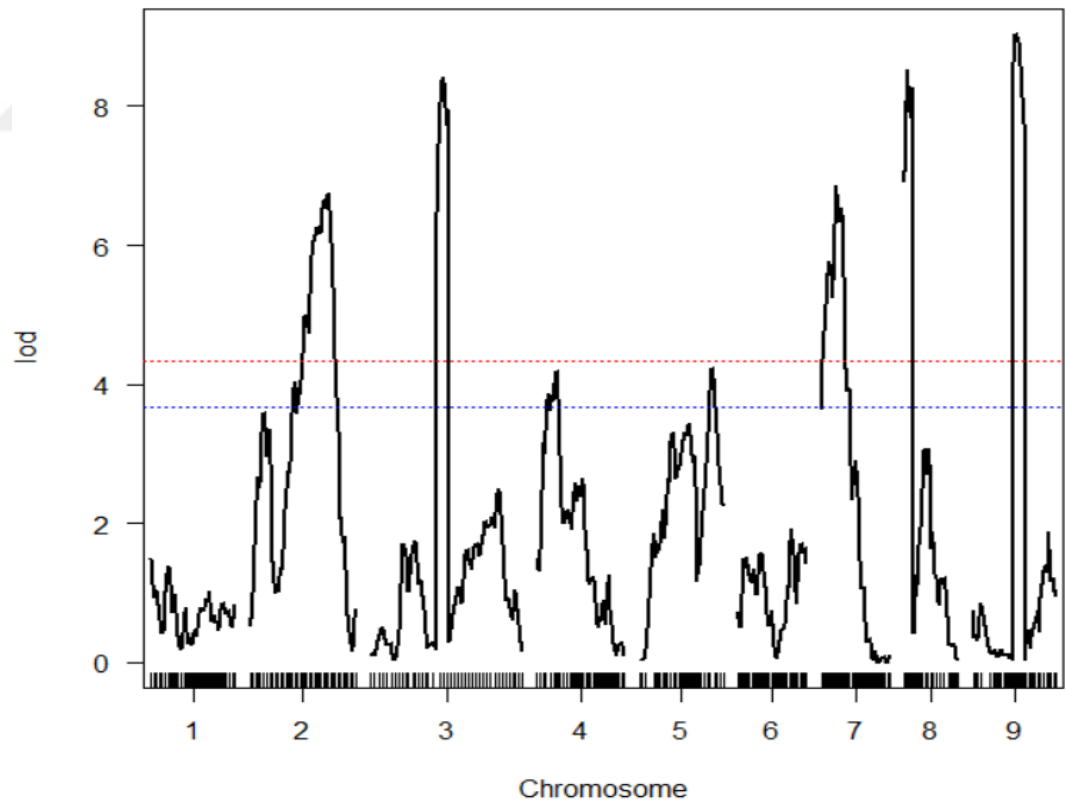


Figure 4.4. Outer Leaf length: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/ctl.

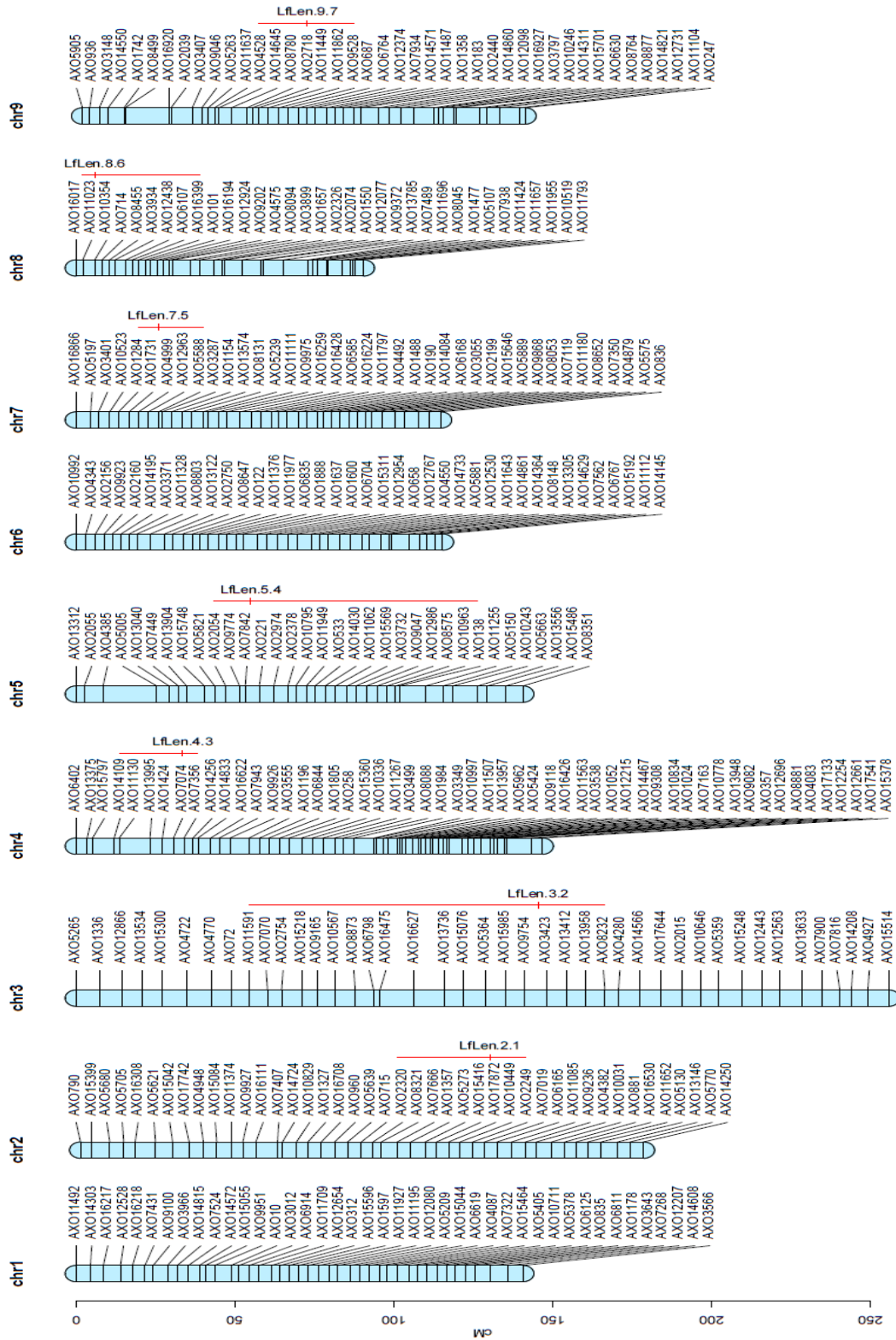


Figure 4.5. Cabbage Linkage map with marked QTLs for Outer Leaf Length.

4.3 Outer Leaf Width

The LOD score threshold at 5% for the Outer Leaf width was 3.42 and for 1% was 4.02 (Table 4.5). After performing Composite Interval Mapping (CIM), five putative QTLs were detected on chromosomes 2, 4, 7, 8, and 9 (Table 4.5), and they had LOD scores higher than 3.42 (Figure 4.6). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 2 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Outer Leaf width. Final result were six putative QTLs at the following positions: at 15.86 cM on chromosome 2, at 128.61 cM on chromosome 2, at 12.02 cM on chromosome 4, at 25.95 cM on chromosome 7, at 5.88 cM on chromosome 8 and at 62.47 cM on chromosome 9 (Table 4.6). Multiple QTL analyses model explains for the 20.34% of the phenotypical variation (Table 4.6). There was no major QTLs. R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.7).

Table 4.5. Outer Leaf width: (left) LOD thresholds, (right) Initial CIM results; R/ql

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.42	c1.loc120	1	120.00	2.02
1%	4.02	AXO5705	2	15.00	4.51
		AXO12443	3	215.89	2.37
		c4.loc12.5	4	12.50	4.79
		c5.loc37.5	5	37.50	2.78
		AXO11112	6	113.20	2.54
		AXO5588	7	25.95	8.28
		AXO10354	8	5.88	6.38
		c9.loc61	9	62.97	8.45

Table 4.6. Outer Leaf width: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model and a P value; R/qrtl

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
LfWid.2.1	2	15.86	6.99	3.27	1.34E-07
LfWid.2.2	2	128.61	5.18	2.42	7.93E-06
LfWid.4.3	4	12.02	5.23	2.44	7.14E-06
LfWid.7.4	7	25.95	10.02	4.74	1.37E-10
LfWid.8.5	8	5.88	7.36	3.45	5.78E-08
LfWid.9.6	9	62.47	8.55	4.02	3.86E-09
TOTAL			43.33	20.34	

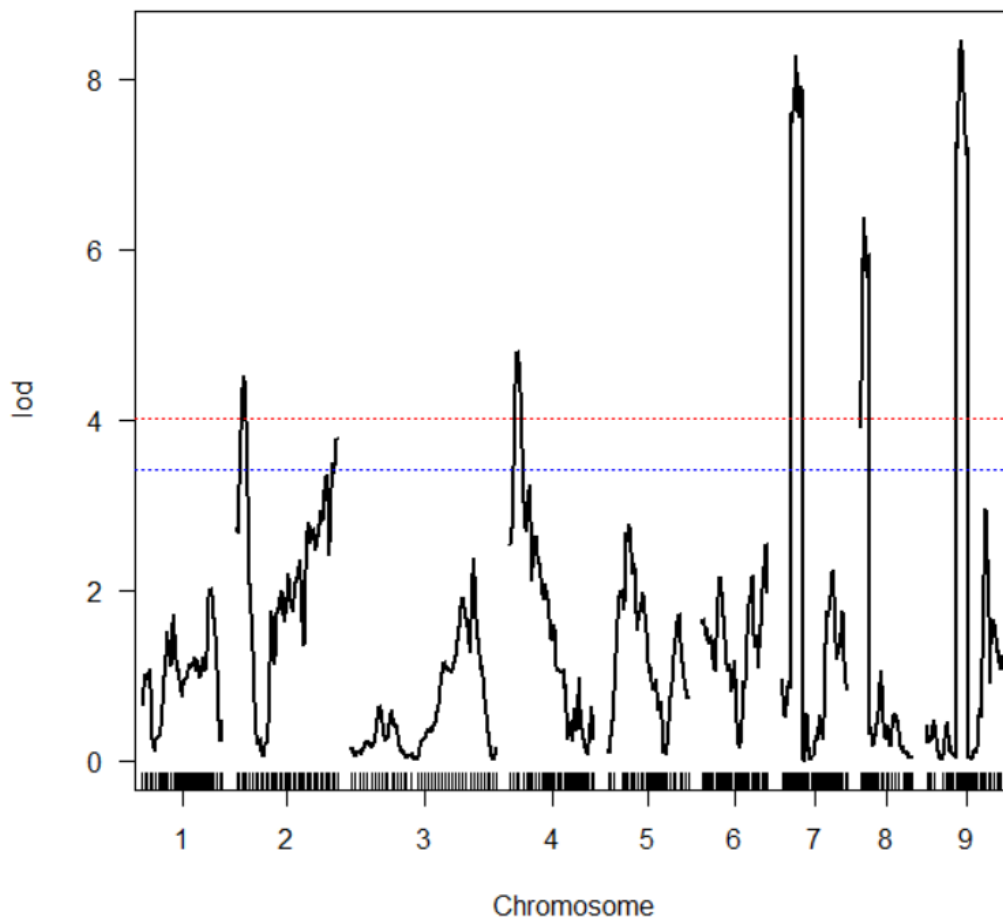


Figure 4.6. Outer Leaf width: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/qrtl.



4.4 Leaf Erectness

The LOD score threshold at 5% for the Leaf erectness was 3.54 and for 1% was 4.15 (Table 4.7). After performing Composite Interval Mapping (CIM), four putative QTLs were detected on chromosomes 1, 2, 3, and 7 (Table 4.7), and they had LOD scores higher than 3.54 (Figure 4.8). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. No additional QTL positions were found, and existing QTL positions were refined. There was no epistasis among the QTLs for the Leaf erectness. Final result were four putative QTLs at the following positions: at 93.50 cM on chromosome 1, at 21.86 cM on chromosome 2, at 51.00 cM on chromosome 3 and at 37.50 cM on chromosome 7, (Table 4.8.). Multiple QTL analysis model explains for the 17.54% of the phenotypical variation (Table 4.8). There are no major QTLs. R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.9).

Table 4.7. Leaf erectness: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.54	c1.loc93.5	1	93.50	6.55
1%	4.15	c2.loc20.5	2	21.90	14.28
		c3.loc50.5	3	50.50	11.9
		c4.loc8	4	8.00	3.20
		AXO8575	5	97.90	2.79
		AXO14145	6	115.40	1.48
		c7.loc36.5	7	36.50	5.69
		c8.loc40.5	8	40.50	1.57
		c9.loc109	9	111.00	1.50

Table 4.8. Leaf erectness: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model and a P value R/ql

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
LfEre.1.1	1	93.50	6.69	2.99	2.35E-07
LfEre.2.2	2	21.86	14.13	6.5	1.04E-14
LfEre.3.3	3	51.00	11.95	5.42	1.49E-12
LfEre.7.4	7	37.50	5.9	2.63	1.49E-06
TOTAL			38.67	17.54	

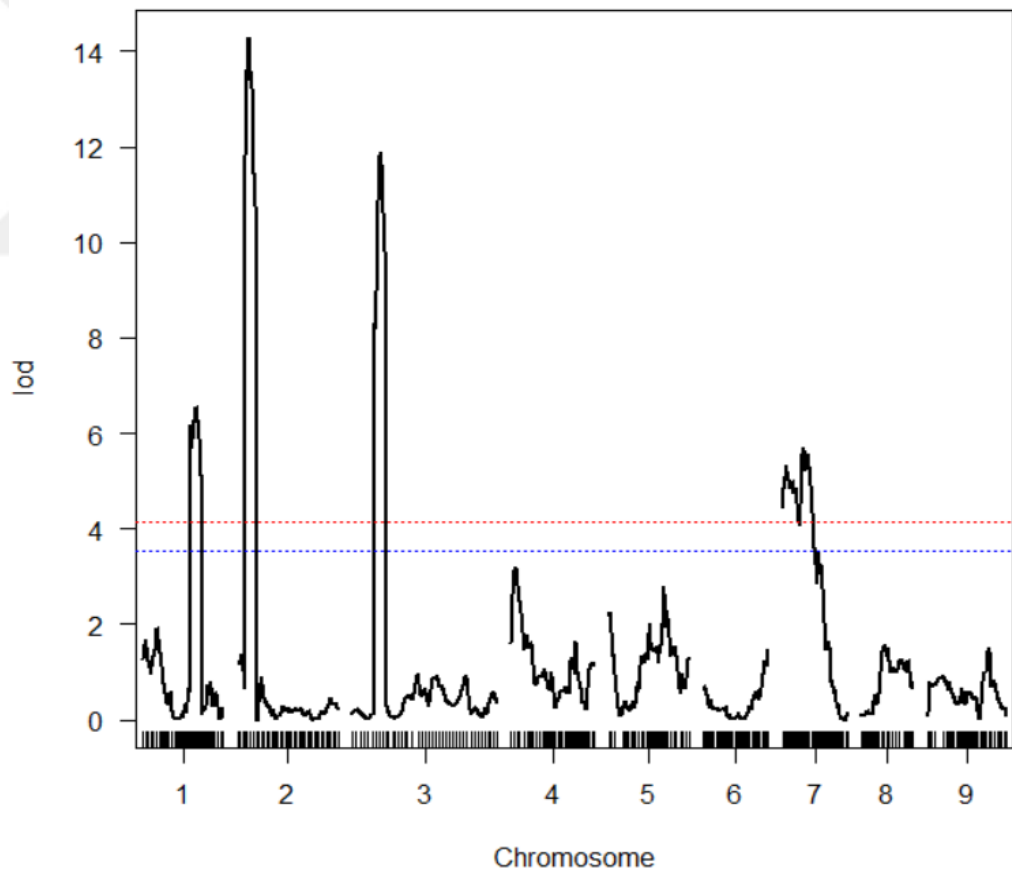


Figure 4.8. Leaf Erectness: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/ql.

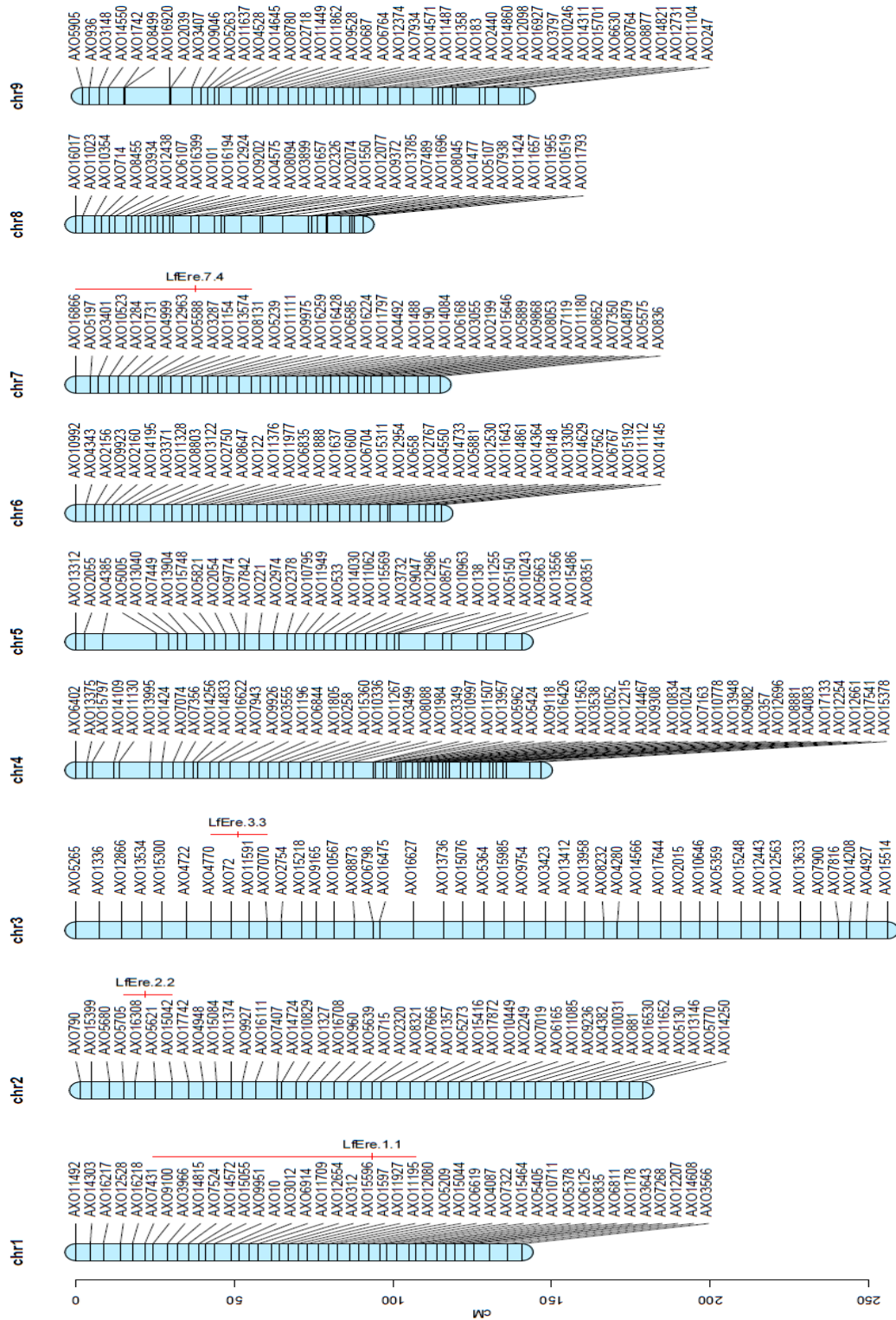


Figure 4.9. Cabbage Linkage map with marked QTLs for Leaf Erectness.

4.5 Head weight

The LOD score threshold at 5% for the Head weight was 3.40 and for 1% was 4.02 (Table 4.9). After performing Composite Interval Mapping (CIM), five putative QTLs were detected on chromosomes 1, 2, 3, 5, and 8 (Table 4.9), and they had LOD scores higher than 3.40 (Figure 4.10). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 2 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Head weight. Final result were six putative QTLs at the following positions: at 101.00 cM on chromosome 1, at 67.36 cM on chromosome 2, at 22.36 cM on chromosome 2, at 139.50 cM on chromosome 3 and at 4.50 cM on chromosome 8 (Table 4.10). Multiple QTL analyses model explains for the 26.23% of the phenotypical variation (Table 4.10). There was no major QTLs. R function 'lmv.linkage.plot' was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.11).

Table 4.9. Head Weight: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.40	AXO5405	1	101.10	5.89
1%	4.02	c2.loc63	2	64.40	16.58
		c3.loc145	3	145.00	8.34
		AXO7943	4	45.10	2.91
		AXO10963	5	100.60	9.74
		AXO122	6	36.70	1.76
		AXO16259	7	47.50	2.9
		c8.loc5	8	5.00	3.41
		AXO3797	9	106.50	1.30

Table 4.10. Head Weight: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model and a P value; R/ql

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
HdWei.1.1	1	101.00	6.69	2.66	2.16E-07
HdWei.2.2	2	67.36	21.5	8.91	< 2E-16
HdWei.2.3	2	22.36	14.06	5.07	1.47E-14
HdWei.3.4	3	139.50	9.9	4	1.47E-10
HdWei.5.5	5	100.57	9.5	3.79	4.73E-10
HdWei.8.6	8	4.50	4.45	1.8	3.30E-05
TOTAL:			66.1	26.23	

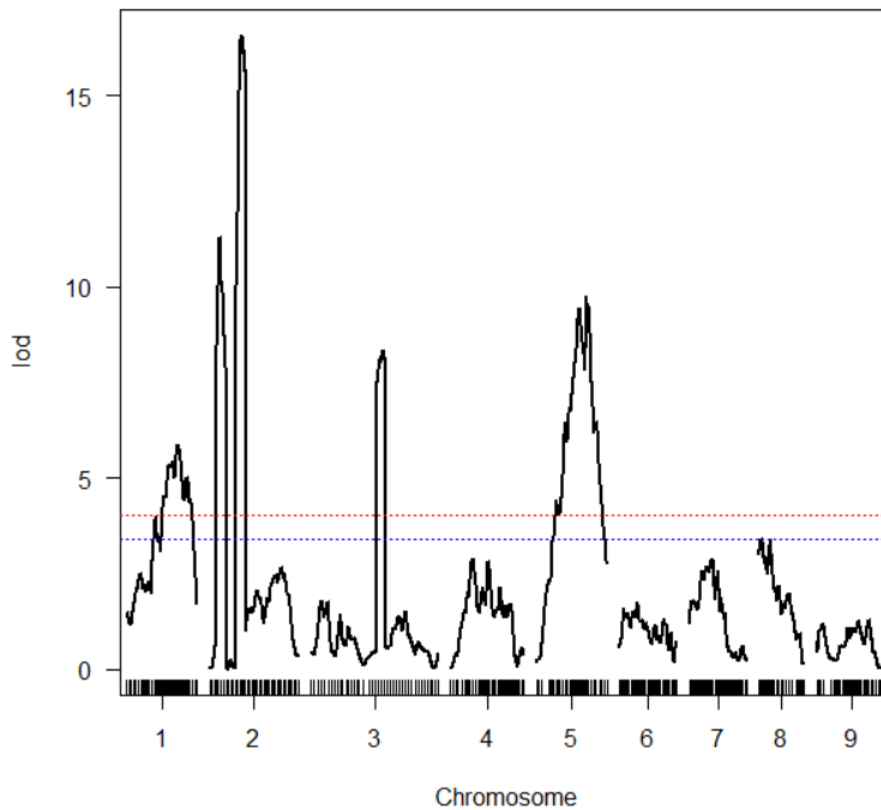


Figure 4.10. Head weight: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/ql.

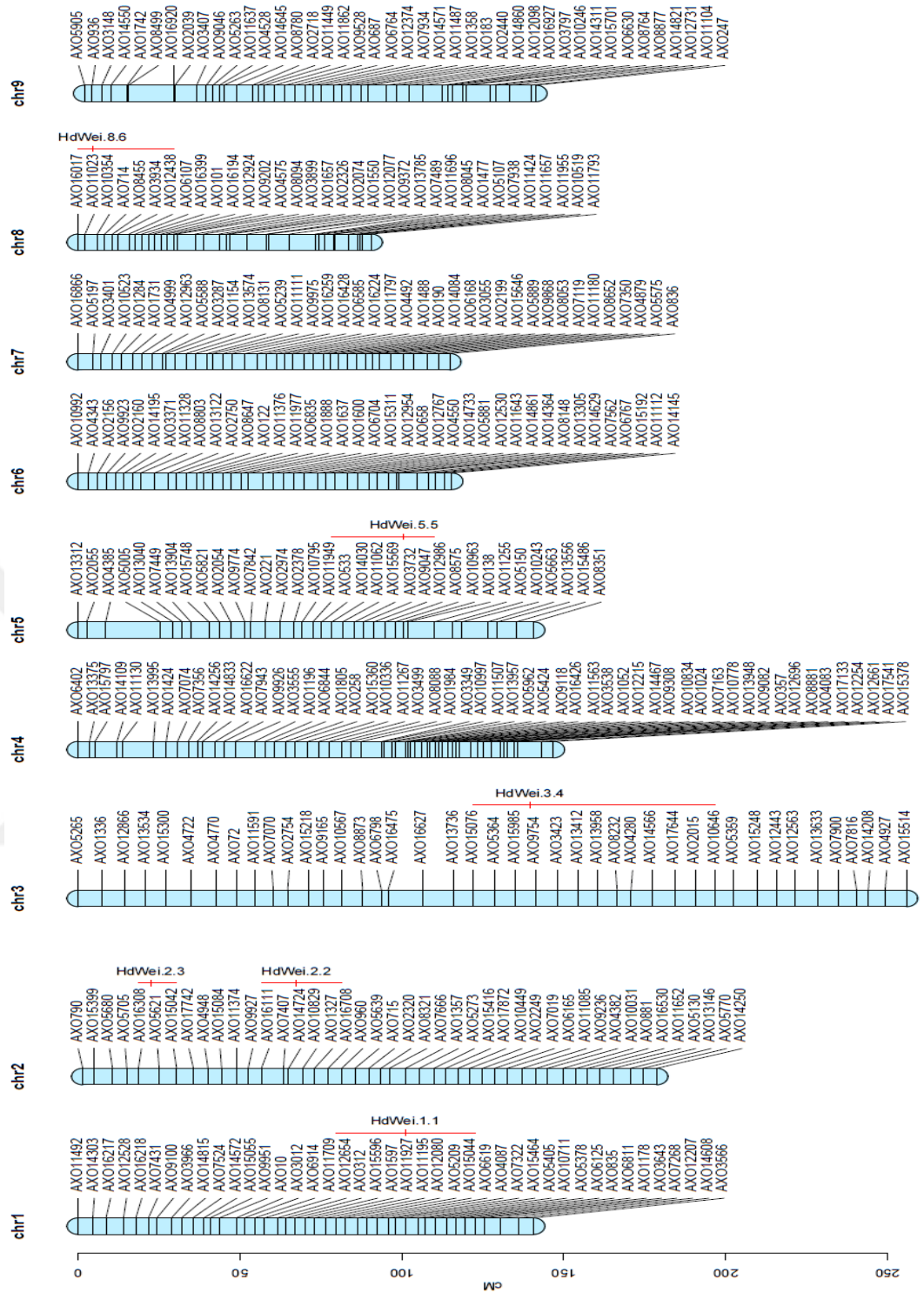


Figure 4.11. Cabbage Linkage map with marked QTLs for Head Weight.

4.6 Head Width

The LOD thresholds at 5% for Head width was 3.28 and for 1% was 4.11. For Stem length LOD score threshold at 5% was 3.28 and for 1% was 4.11 (Table 4.11). After performing Composite Interval Mapping (CIM), three putative QTLs were detected on chromosomes 2, 3, and 5 (Table 4.11), and they had LOD scores higher than 3.28 (Figure 4.12). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 2 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Head width. Final result were three putative QTLs at the following positions: at 26.86 cM on chromosome 2, at 163.86 cM on chromosome 2, at 139.50 cM on chromosome 3 and at 106.50 cM on chromosome 5 (Table 4.12). Multiple QTL analyses model explains for the 19.32% of the phenotypical variation (Table 4.12). No major QTLs were found. R function 'lmv.linkage.plot' was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.13).

Table 4.11. Head width: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.28	AXO4087	1	92.25	1.735
1%	4.11	c2.loc162	2	163.36	9.837
		c3.loc144	3	144.00	9.621
		c4.loc79	4	79.00	1.08
		AXO10963	5	100.57	4.308
		AXO9923	6	8.93	1.701
		AXO16259	7	47.55	2.311
		AXO16017	8	0.00	2.622
		AXO1742	9	15.11	0.67

Table 4.12. Head width: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model and a P value; R/qlt

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
HdWid.2.1	2	26.86	7.7	4.4	2.52E-08
HdWid.2.2	2	163.86	10.4	6.04	5.32E-11
HdWid.3.3	3	139.50	10.8	6.28	2.19E-11
HdWid.5.4	5	106.50	4.55	2.6	3.21E-05
TOTAL:			33.45	19.32	

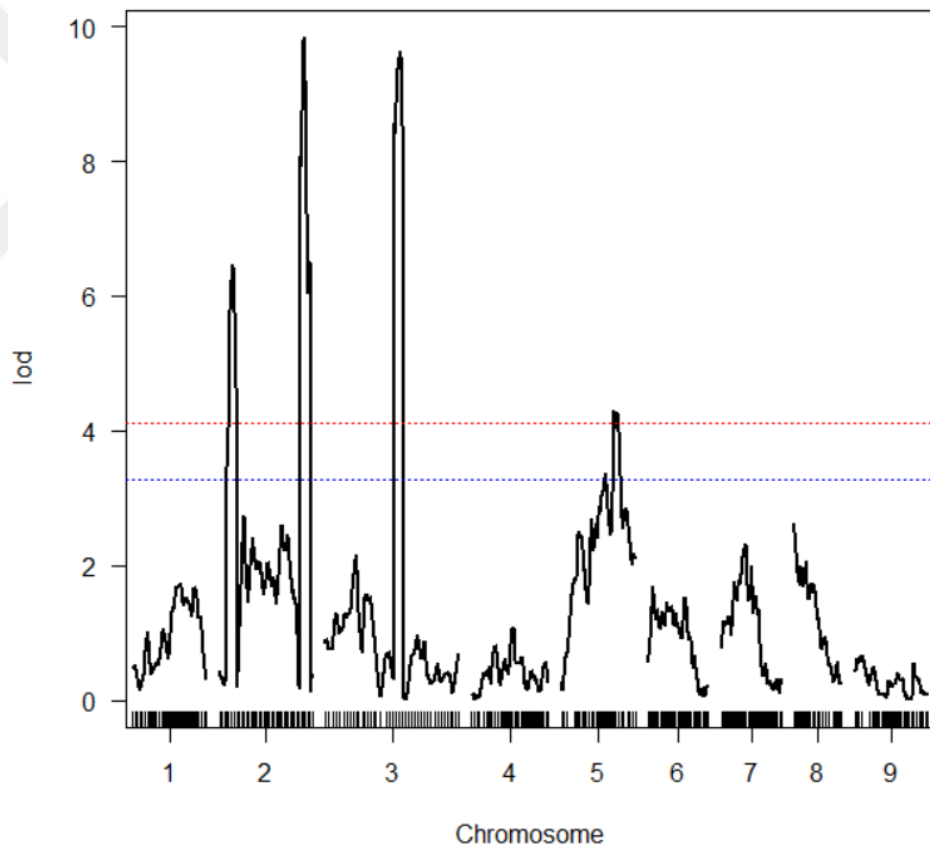


Figure 4.12. Head width: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/qlt.

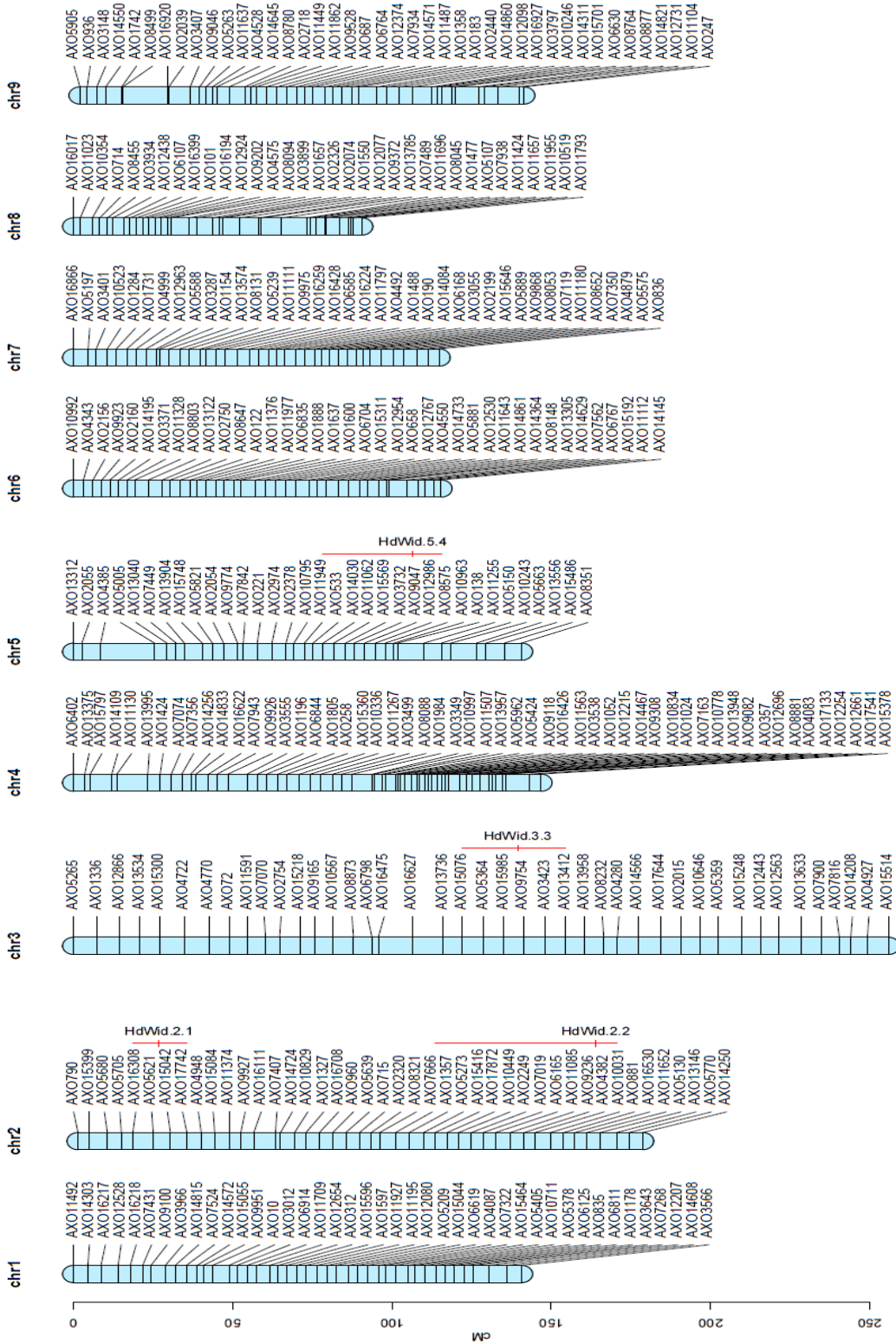


Figure 4.13. Cabbage Linkage map with marked QTLs for Head width.

4.7 Head Length

The LOD score threshold at 5% for the Head length was 3.37 and for 1% was 4.35 (Table 4.13). After performing Composite Interval Mapping (CIM), three putative QTLs were detected on chromosomes 2, 6, and 7 (Table 4.13), and they had LOD scores higher than 3.37 (Figure 4.14). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 2 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Head length. Final result was four putative QTLs at the following positions: at 18.61 cM on chromosome 2, at 95.86 cM on chromosome 2, at 113.20 cM on chromosome 6 and at 25.95 cM on chromosome 7 (Table 4.14). Multiple QTL analyses model explains for the 31.97% of the phenotypical variation with HdLen.2.1 being the major QTL which explains 17.10% of variation with LOD score of 32.74 (Table 4.14). Function ‘effectplot’ was used to plot the phenotype averages for the genotype groups at HdLen.2.1 QTL marker position (Figure 4.15). R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.16).

Table 4.13. Head length: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.37	c1.loc94	1	94.00	2.06
1%	4.35	c2.loc18.5	2	19.90	38.04
		AXO16475	3	95.70	1.63
		AXO1805	4	64.20	1.68
		AXO13904	5	34.80	1.88
		AXO11112	6	113.20	3.42
		AXO5588	7	26.00	6.48
		c8.loc2.5	8	2.50	1.25
		AXO12098	9	98.40	1.39

Table 4.14. Head length: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model, P value and a major QTL (in bold); R/qlt

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
HdLen.2.1	2	18.61	32.73	17.10	< 2E-16
HdLen.2.2	2	95.86	19.24	9.60	<2E-16
HdLen.6.3	6	113.20	3.58	1.69	0.000294
HdLen.7.4	7	25.95	7.47	3.58	4.24E-08
TOTAL:			63.02	31.97	

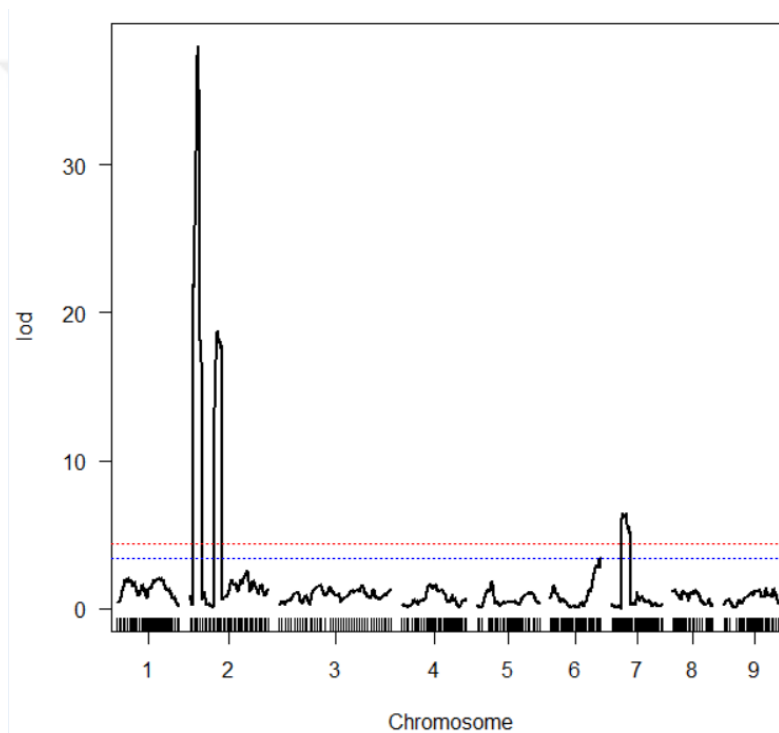


Figure 4.14. Head length: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/qlt.

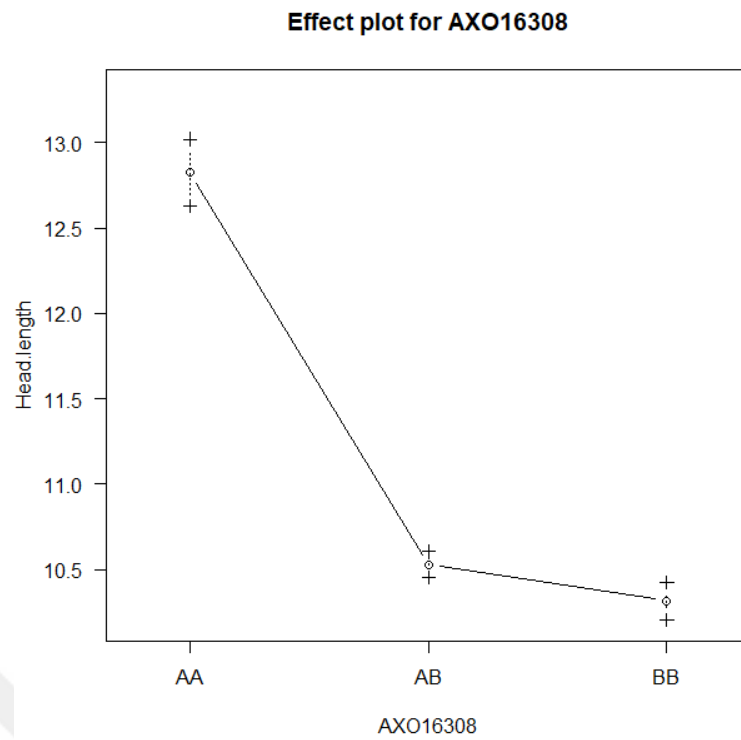


Figure 4.15. Head length: Effect plot for the major QTL HdLen.2.1.

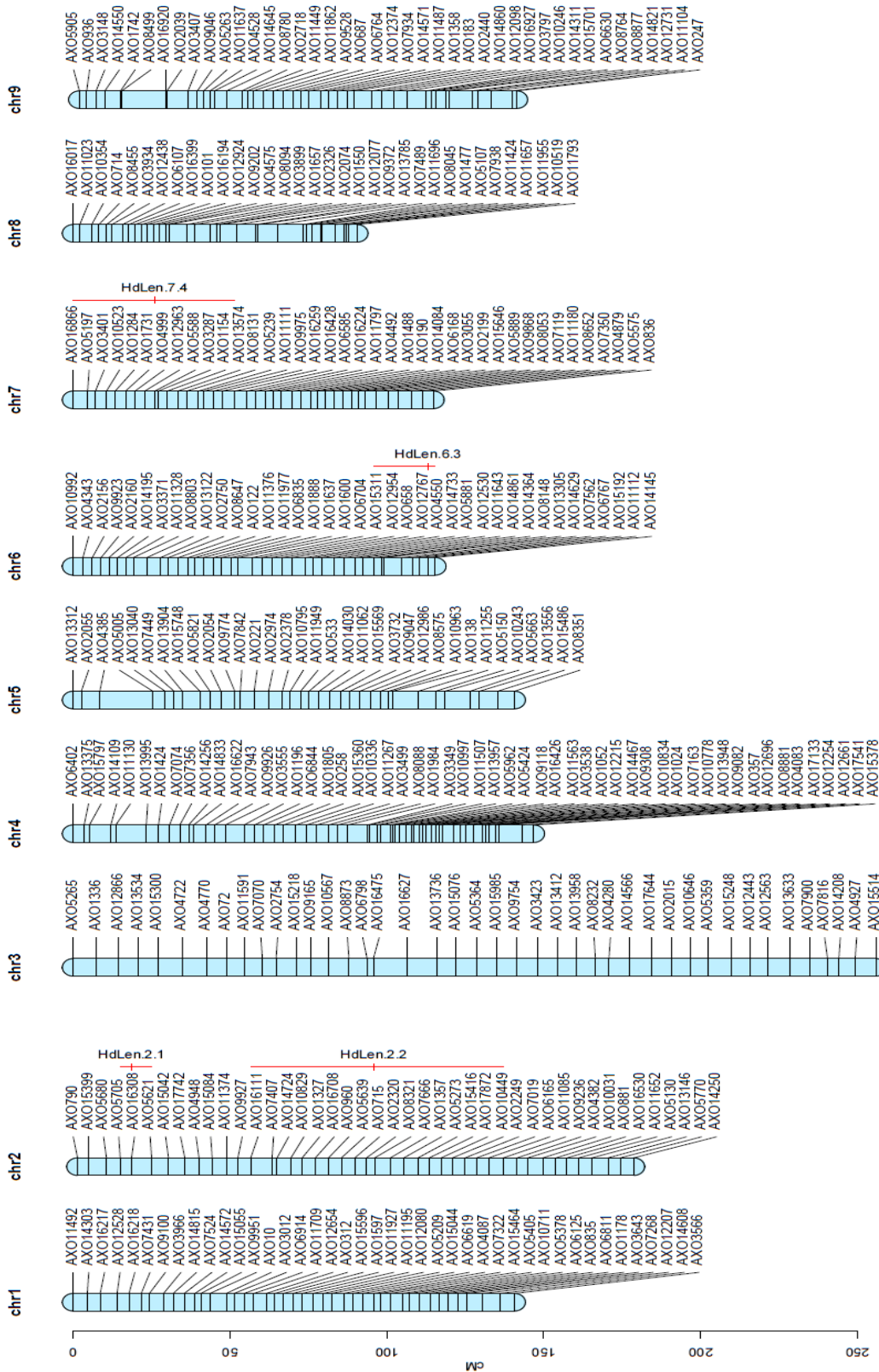


Figure 4.16. Cabbage Linkage map with marked QTLs for Head Length.

4.8 Head Shape (HH/HW Ratio)

The LOD score threshold at 5% for the Head shape was 3.57 and for 1% was 4.40. After performing Composite Interval Mapping (CIM), four putative QTLs were detected on chromosomes 2, 3, 5, and 6 (Table 4.15), and they had LOD scores higher than 3.57 (Figure 4.17). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 3 and thus added to the model which was tested again. Then QTL positions were refined. Epistasis was found among the QTLs for the Head shape (Figure 4.18). Final result were five putative QTLs at the following positions: at 19.36 cM on chromosome 2, at 61.00 cM on chromosome 3, at 150.00 cM on chromosome 3, at 132.50 cM on chromosome 5 and at 108.15 cM on chromosome 6 (Table 4.16). Multiple QTL analyses model explains for the 46.45% of the phenotypical variation with HdRat.2.1 being the major QTL with 37.09% of variation explained (Table 4.16). Function ‘effectplot’ was used to plot the phenotype averages for the genotype groups at HdRat.2.1 QTL marker position (Figure 4.19). R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.20).

Table 4.15. Head shape: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.57	c1.loc22.5	1	22.50	1.056
1%	4.40	c2.loc19	2	20.40	87.881
		c3.loc158	3	158.00	8.535
		c4.loc142	4	142.00	0.98
		c5.loc132	5	132.00	4.836
		AXO6767	6	108.20	5.328
		AXO16866	7	0.00	1.927
		AXO11793	8	90.50	0.982
		c9.loc40.5	9	42.50	1.86

Table 4.16. Head shape: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model, P value and a major QTL (in bold); R/qtl

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
HdRat.2.1	2	19.36	76.07	37.09	< 2e-16
HdRat.3.2	3	61.00	6.45	2.46	4.53E-07
HdRat.3.3	3	150.00	8.53	3.27	4.06E-09
HdRat.5.4	5	132.50	4.63	1.75	2.82E-05
HdRat.6.5	6	108.15	4.95	1.88	1.35E-05
TOTAL:			100.63	46.45	

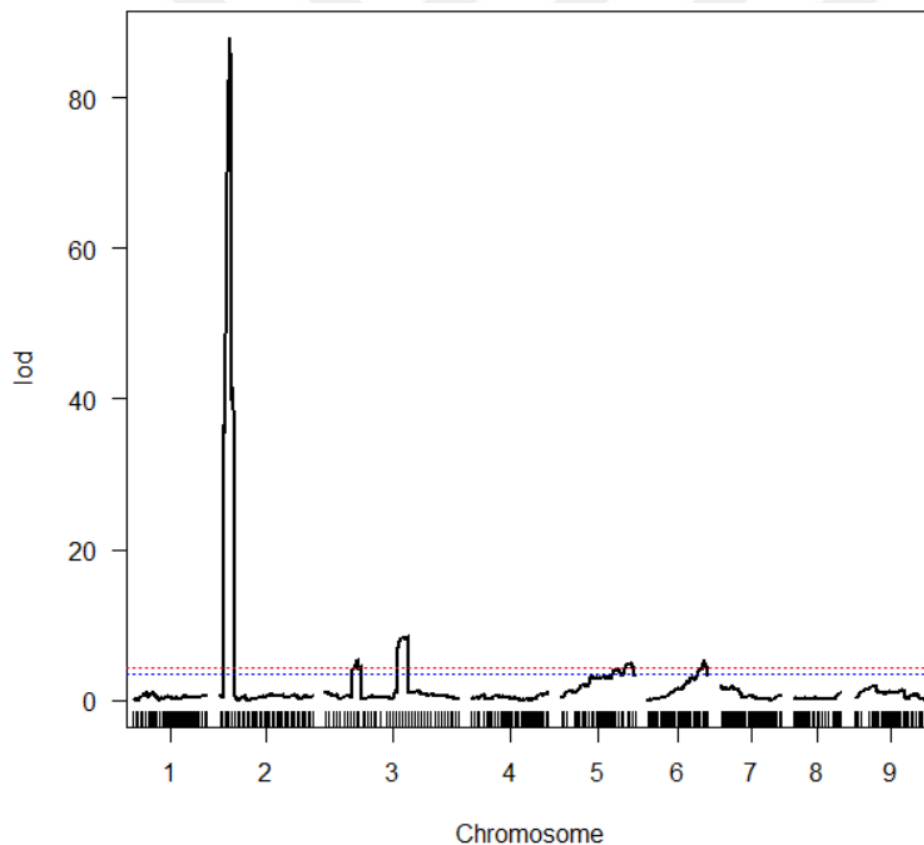


Figure 4.17. Head shape: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/qtl.

	Pvalue (Chi2)	Pvalue (F)	
2@20.4	0.000	< 2e-16	***
3@61.0	0.000	3.11e-15	***
3@158.0	0.000	4.76e-14	***
5@132.0	0.000	4.09e-14	***
6@108.2	0.000	0.000507	***
2@20.4:3@61.0	0.000	6.79e-11	***
2@20.4:3@158.0	0.000	7.54e-07	***
2@20.4:5@132.0	0.000	2.94e-09	***
2@20.4:6@108.2	0.000	0.004169	**
3@61.0:3@158.0	0.000	0.000110	***
3@61.0:5@132.0	0.000	1.19e-07	***
3@61.0:6@108.2	0.000	0.205639	
3@158.0:5@132.0	0.000	4.01e-07	***
3@158.0:6@108.2	0.000	0.235288	
5@132.0:6@108.2	0.000	0.000673	***
2@20.4:3@61.0:3@158.0	0.000	0.013961	*
2@20.4:3@61.0:5@132.0	0.000	9.14e-05	***
2@20.4:3@61.0:6@108.2	0.000	0.056832	.
2@20.4:3@158.0:5@132.0	0.000	0.002375	**
2@20.4:3@158.0:6@108.2	0.003	0.342750	
2@20.4:5@132.0:6@108.2	0.000	0.000891	***
3@61.0:3@158.0:5@132.0	0.000	0.011816	*
3@61.0:3@158.0:6@108.2	0.184	0.897878	
3@61.0:5@132.0:6@108.2	0.000	0.158998	
3@158.0:5@132.0:6@108.2	0.000	0.051508	.
2@20.4:3@61.0:3@158.0:5@132.0	0.018	0.477527	
2@20.4:3@61.0:3@158.0:6@108.2	0.113	0.766827	
2@20.4:3@61.0:5@132.0:6@108.2	0.000	0.135318	
2@20.4:3@158.0:5@132.0:6@108.2	0.000	0.068438	.
3@61.0:3@158.0:5@132.0:6@108.2	0.034	0.574940	
2@20.4:3@61.0:3@158.0:5@132.0:6@108.2	0.048	0.524404	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1			

Figure 4.18. Head shape: Epistasis significance represented by the number of stars (*), in red rectangle; R/qtl.

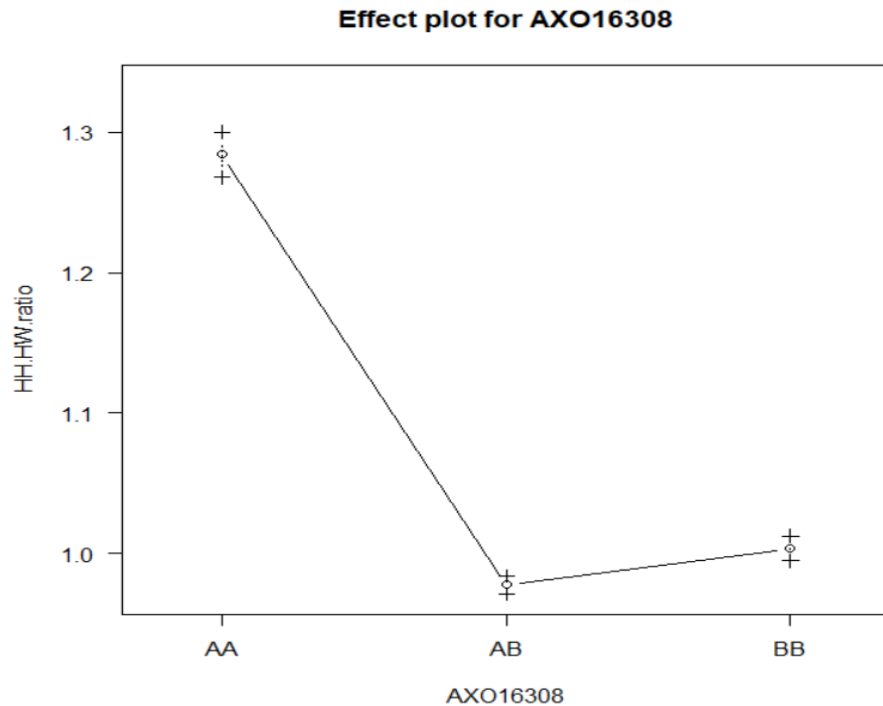


Figure 4.19. Head shape: Effect plot for the major QTL HdRat.2.1.

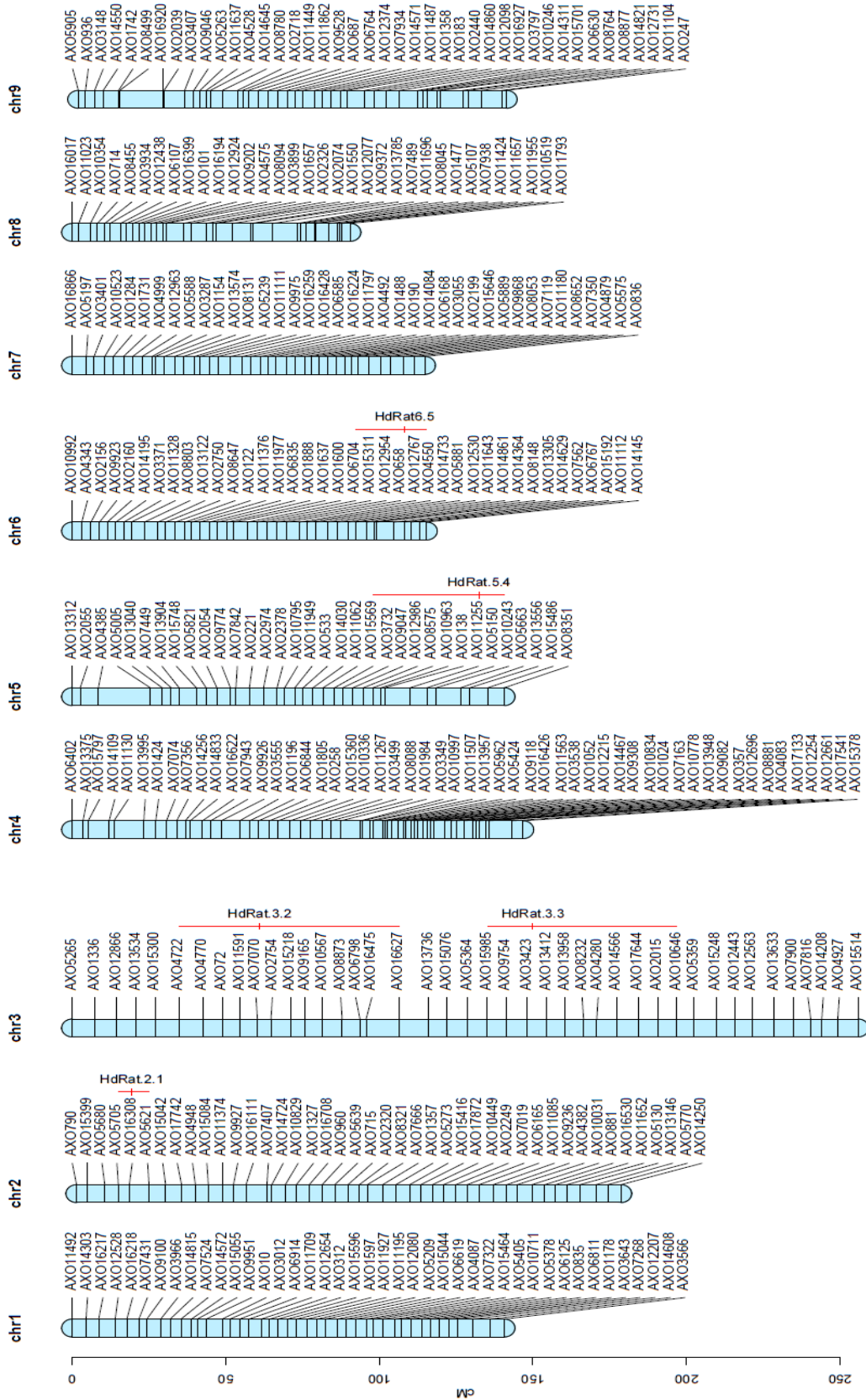


Figure 4.20. Cabbage Linkage map with marked QTLs for Head shape.

4.9 Head Density

The LOD score threshold at 5% for the Head density was 3.51 and for 1% was 4.45 (Table 4.17). After performing Composite Interval Mapping (CIM), five putative QTLs were detected on chromosomes 1, 2, 4, 5, and 6 (Table 4.17), and they had LOD scores higher than 3.51 (Figure 4.21). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Two additional QTLs were found on the chromosome 2 and 4 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Head density. Final result were seven putative QTLs at the following positions: at 78.50 cM on chromoson 1, at 18.86 cM on chromosome 2, at 117.26 cM on chromosome 2, at 13.61 cM on chromosome 4, at 146.79 cM on chromosome 4, at 102.50 cM on chromosome 5 and at 44.74 cM on chromosome 6 (Table 4.18). Multiple QTL analyses model explains for the 37.37% of the phenotypical variation with HdDen.2.2 being the major QTL with 21.67% of variation explained (Table 4.18). Function ‘effectplot’ was used to plot the phenotype averages for the genotype groups at HdDen.2.2 QTL marker position (Figure 4.22). R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.23).

Table 4.17. Head density: (left) LOD thresholds, (right) Initial CIM results; R/ql

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.51	c1.loc80	1	80.00	8.92
1%	4.45	c2.loc17.5	2	18.90	42.72
		c3.loc57	3	57.00	2.75
		c4.loc146	4	146.00	3.96
		AXO138	5	101.80	4.87
		c6.loc46	6	46.00	4.56
		AXO9868	7	88.70	1.64
		AXO16399	8	19.70	3
		AXO14311	9	114.10	2.48

Table 4.18. Head density: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model, P value and a major QTL (in bold); R/qlt

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
HdDen.1.1	1	78.50	12.07	5.28	1.58E-12
HdDen.2.2	2	18.86	44.29	21.67	< 2E-16
HdDen.2.3	2	117.26	5.57	2.38	3.57E-06
HdDen.4.4	4	13.61	4.57	1.95	3.41E-05
HdDen.4.5	4	146.79	4.3	1.81	6.84E-05
HdDen.5.6	5	102.50	4.77	2.04	2.15E-05
HdDen.6.7	6	44.74	5.24	2.24	7.45E-06
TOTAL:			80.81	37.37	

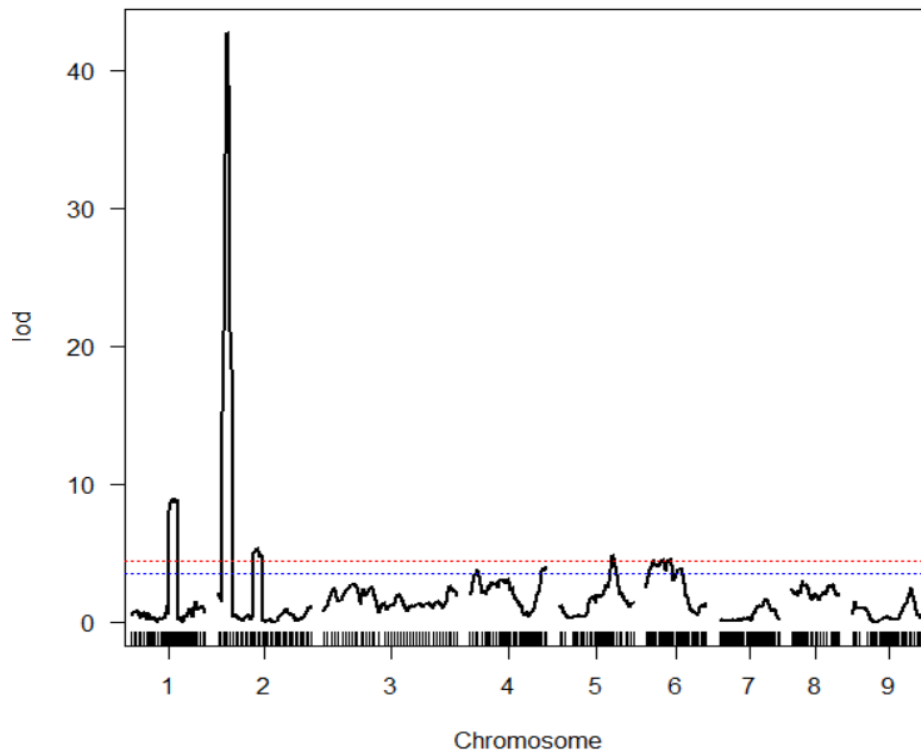


Figure 4.21. Head density: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/qlt.

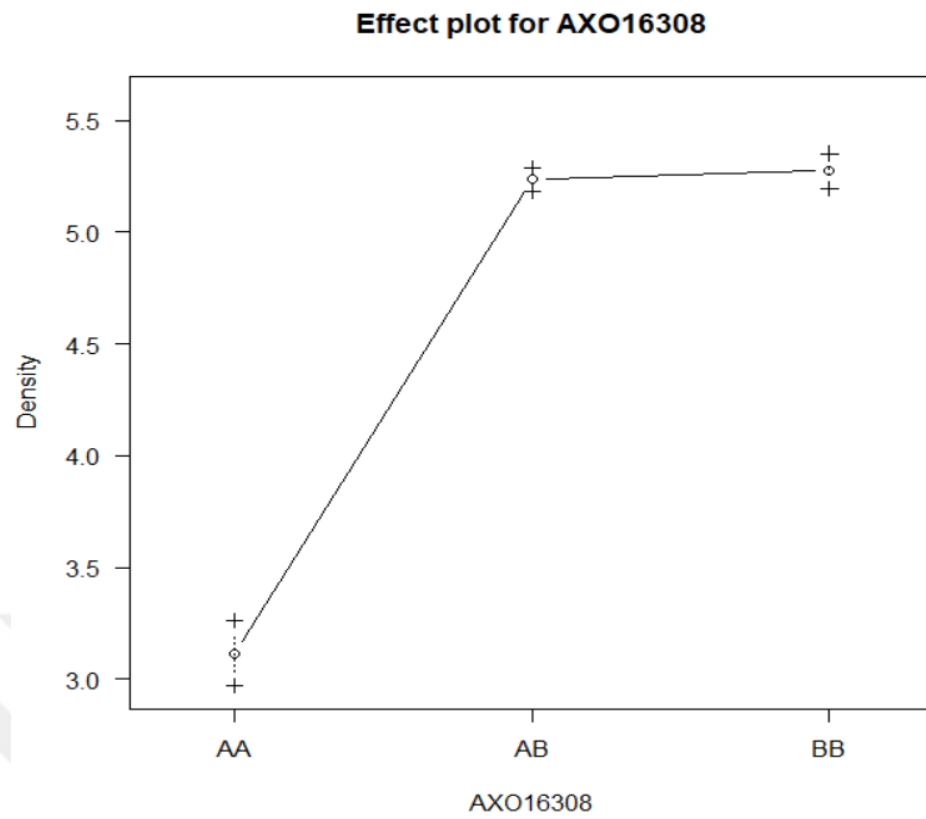


Figure 4.22. Head density: Effect plot for the major QTL HdDen.2.2.



4.10 Colour

The LOD score threshold at 5% for the Colour was 3.55 and for 1% was 4.09 (Table 4.19). After performing Composite Interval Mapping (CIM), three putative QTLs were detected on chromosomes 2, 3, and 7 (Table 4.19), and they had LOD scores higher than 3.55 (Figure 4.24). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 2 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Colour. Final result were four putative QTLs at the following positions: at 20.86cM on chromoson 2, at 86.86 cM on chromosome 2, at 122.02 cM on chromosome 3 and at 41.67 cM on chromosome 7 (Table 4.20). Multiple QTL analyses model explains for the 23.50% of the phenotypical variation with Col.2.1 being the major QTL with 13.72% of variation explained (Table 4.20). Function ‘effectplot’ was used to plot the phenotype averages for the genotype groups at Col.2.1 QTL marker position (Figure 4.25). R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.26).

Table 4.19. Colour: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.55	AXO6125	1	110.40	1.79
1%	4.09	c2.loc20	2	21.40	36.06
		AXO15076	3	122.00	4.77
		c4.loc37.5	4	37.50	2.63
		AXO7842	5	53.30	1.84
		AXO12767	6	69.70	1.25
		AXO11111	7	41.70	7.11
		AXO1550	8	46.90	2.56
		c9.loc12.5	9	14.50	2.36

Table 4.20. Colour: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model, P value and a major QTL (in bold); R/qlt

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
Col.2.1	2	20.86	33.87	13.72	< 2E-16
Col.2.2	2	86.86	12.01	4.58	1.32E-12
Col.3.3	3	122.02	5.54	2.08	3.33E-06
Col.7.4	7	41.67	8.27	3.12	6.58E-09
TOTAL:			59.69	23.50	

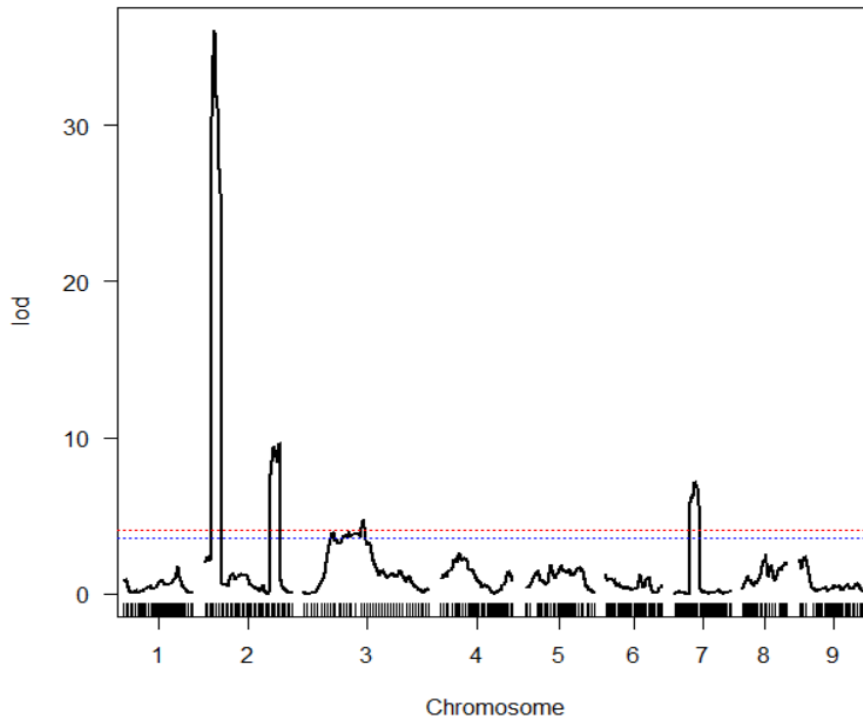


Figure 4.24. Colour: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/qlt.

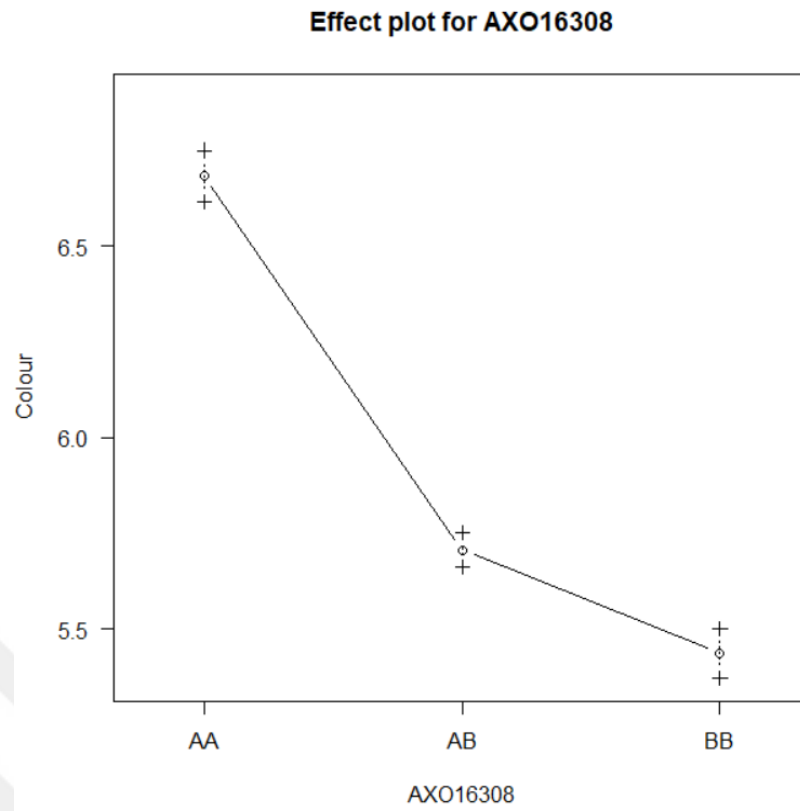


Figure 4.25. Colour: Effect plot for the major QTL Col.2.1.

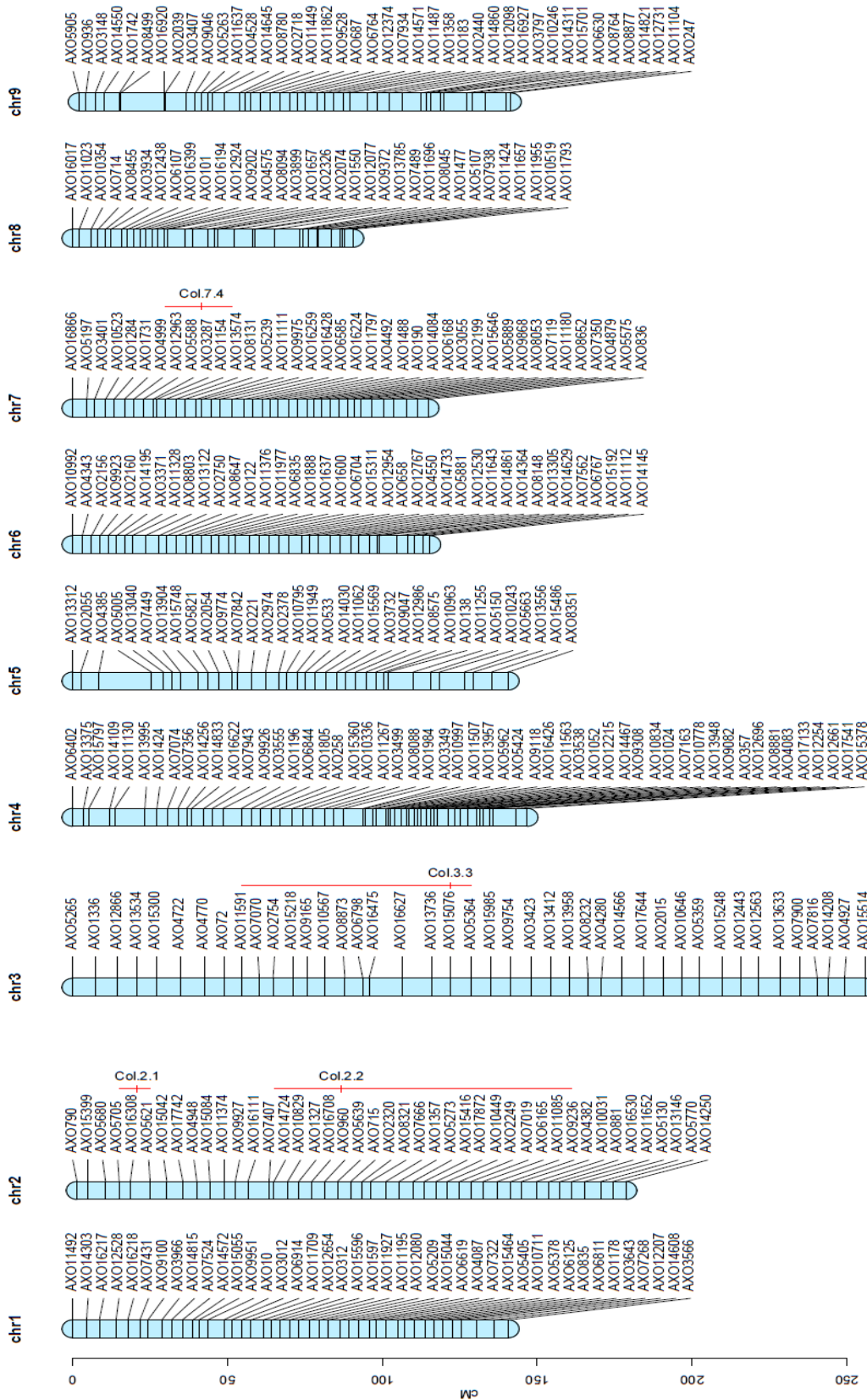


Figure 4.26. Cabbage Linkage map with marked QTLs for Colour.

4.11 Anthocyanin

The LOD score threshold at 5% for the Anthocyanin was 3.46 and for 1% was 4.28 (Table 4.21). After performing Composite Interval Mapping (CIM), four putative QTLs were detected on chromosomes 1, 2, 4, and 7 (Table 4.21), and they had LOD scores higher than 3.46 (Figure 4.27). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 2 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Anthocyanin. Final result was five putative QTLs at the following positions: at 50.50 cM on chromosome 1, at 16.86 cM on chromosome 2, at 68.36 cM on chromosome 2, at 3.45 cM on chromosome 4 and at 74.00 cM on chromosome 7 (Table 4.22). Multiple QTL analyses model explains for the 28.48% of the phenotypical variation with Ant.7.5 being the major QTL with 15.07% of variation explained (Table 4.22). Function ‘effectplot’ was used to plot the phenotype averages for the genotype groups at Ant.7.5 QTL marker position (Figure 4.28). R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.29).

Table 4.21. Anthocyanin: (left) LOD thresholds, (right) Initial CIM results; R/ql

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.46	c1.loc50	1	50.00	7.52
1%	4.28	c2.loc16	2	17.36	6.67
		AXO14566	3	177.30	2.76
		AXO13375	4	3.45	3.72
		AXO8575	5	97.87	1.54
		AXO10992	6	0.00	2.57
		c7.loc74	7	74.00	31.66
		AXO11793	8	90.47	0.95
		AXO247	9	141.41	2.36

Table 4.22. Anthocyanin: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model, P value and a major QTL (in bold); R/ql

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
Ant.1.1	1	50.5	7.22	3.03	7.56E-08
Ant.2.2	2	16.86	9.74	4.13	2.44E-10
Ant.2.3	2	68.36	10.4	4.4	5.47E-11
Ant.4.4	4	3.45	4.43	1.85	4.26E-05
Ant.7.5	7	74.00	33.29	15.07	< 2E-16
TOTAL:			65.08	28.48	

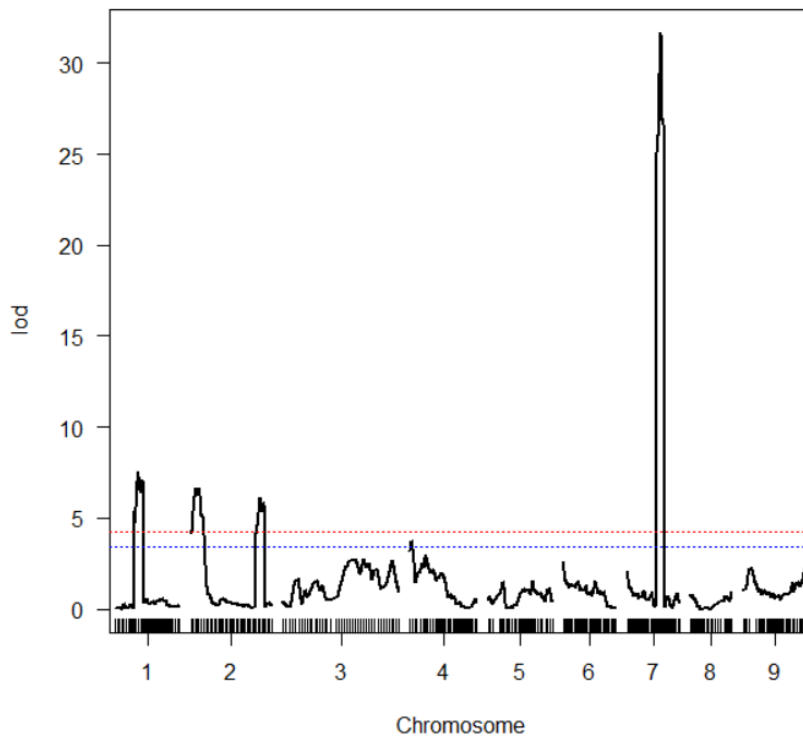


Figure 4.27. Anthocyanin: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/ql.

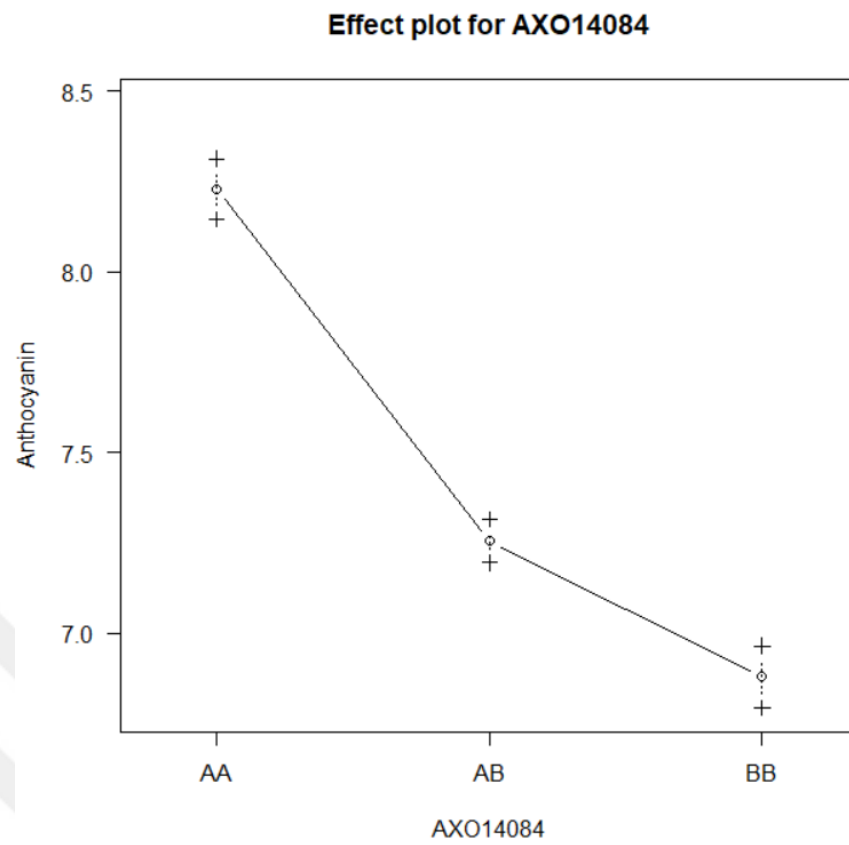


Figure 4.28. Anthocyanin: Effect plot for the major QTL Ant.7.5.

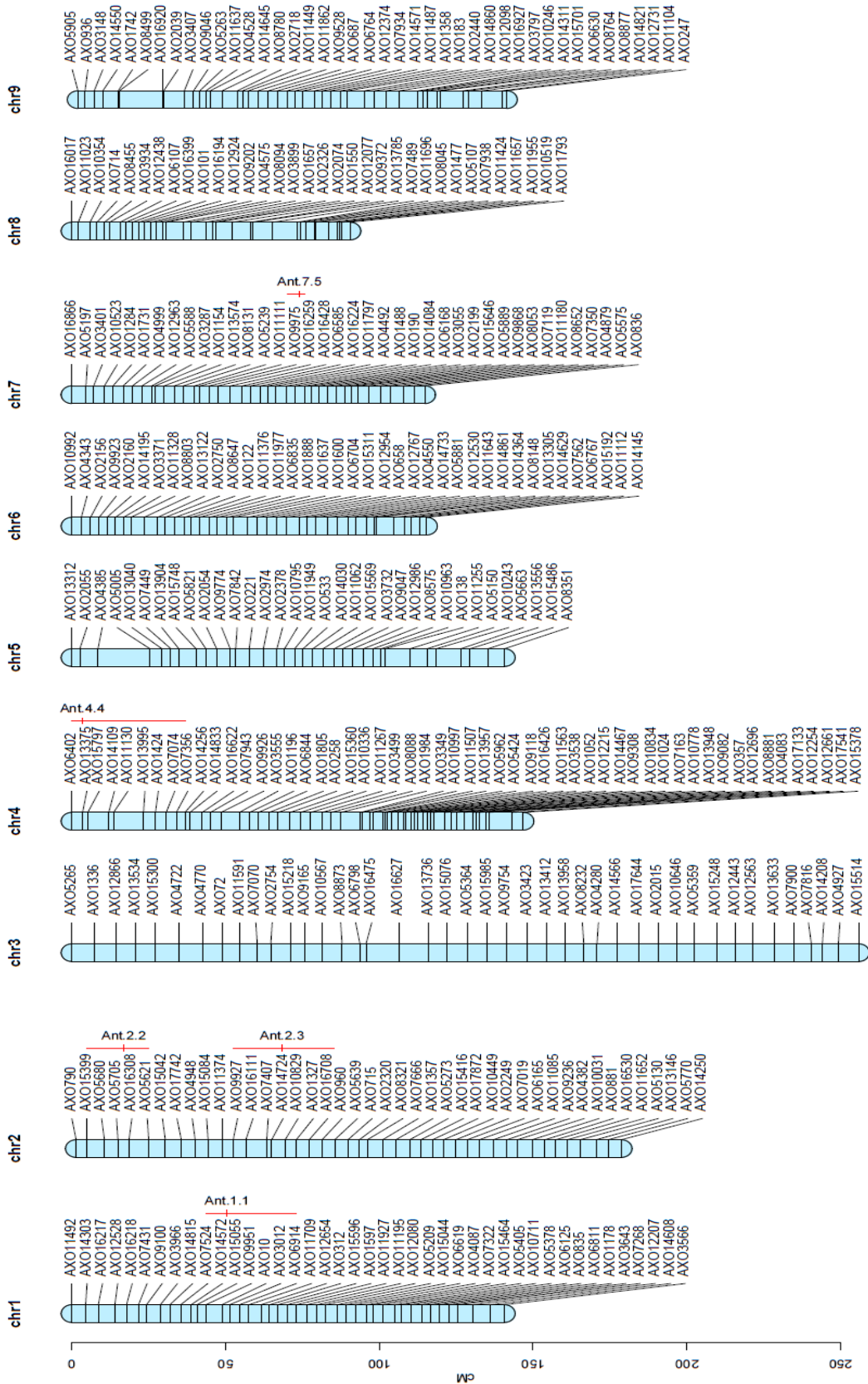


Figure 4.29. Cabbage Linkage map with marked QTLs for Anthocyanin.

4.12 Vigour

The LOD score threshold at 5% for the Vigour was 3.47 and for 1% was 4.30 (Table 4.23). After performing Composite Interval Mapping (CIM), three putative QTLs were detected on chromosomes 2, 6, and 9 (Table 4.23), and they had LOD scores higher than 3.47 (Figure 4.30). After inserting putative QTLs into the genetic map, Multiple QTL analysis was performed. Additional QTL was found on the chromosome 7 and thus added to the model which was tested again. Then QTL positions were refined. There was no epistasis among the QTLs for the Vigour. Final result was four putative QTLs at the following positions: at 18.36 cM on chromosome 2, at 115.38 cM on chromosome 6, at 44.5 cM on chromosome 7, and at 66.78 cM on chromosome 9 (Table 4.24). Multiple QTL analyses model explains for the 27.17% of the phenotypical variation with Vig.2.1 being the major QTL with 20.87% of variation explained (Table 4.24). Function ‘effectplot’ was used to plot the phenotype averages for the genotype groups at Vig.2.1 QTL marker position (Figure 4.31). R function ‘lmv.linkage.plot’ was used to generate linkage map that clearly shows all putative QTLs and their confidentiality intervals (Figure 4.32).

Table 4.23. Vigour: (left) LOD thresholds, (right) Initial CIM results; R/qtl

LOD thresholds	LOD	CIM results			
		(In red: QTLs with LOD higher than 5% threshold)			
		QTL	chromosome	position	LOD
5%	3.47	AXO5378	1	107.30	3.25
1%	4.30	c2.loc18	2	19.40	48.65
		AXO9754	3	141.30	3.02
		c4.loc19	4	19.00	2.33
		AXO10963	5	100.60	2.57
		AXO14145	6	115.40	6.69
		AXO9975	7	44.70	3.45
		AXO16194	8	23.40	3.37
		c9.loc70	9	72.00	5.72

Table 4.24. Vigour: Refined positions, LOD scores of the putative QTLs with percentage of variation explained by the Multiple QTL model, P value and a major QTL (in bold); R/ql

QTL	Chromosome	position in cM	LOD	Variation %	P value (F)
Vig.2.1	2	18.36	48.25	20.87	< 2E-16
Vig.6.2	6	115.38	6.56	2.53	3.25E-07
Vig.7.3	7	44.50	3.67	1.40	0.000235
Vig.9.4	9	66.78	6.15	2.37	8.22E-07
TOTAL:			64.63	27.17	

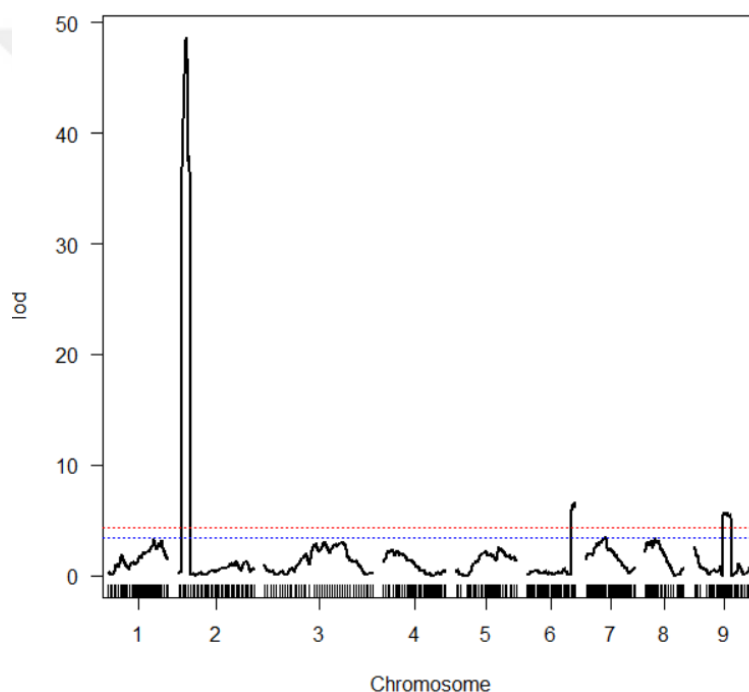


Figure 4.30. Vigour: Position of QTLs on the chromosome with LOD score; blue dotted line signifies 5% LOD threshold, red dotted line signifies 1% LOD threshold; R/ql.

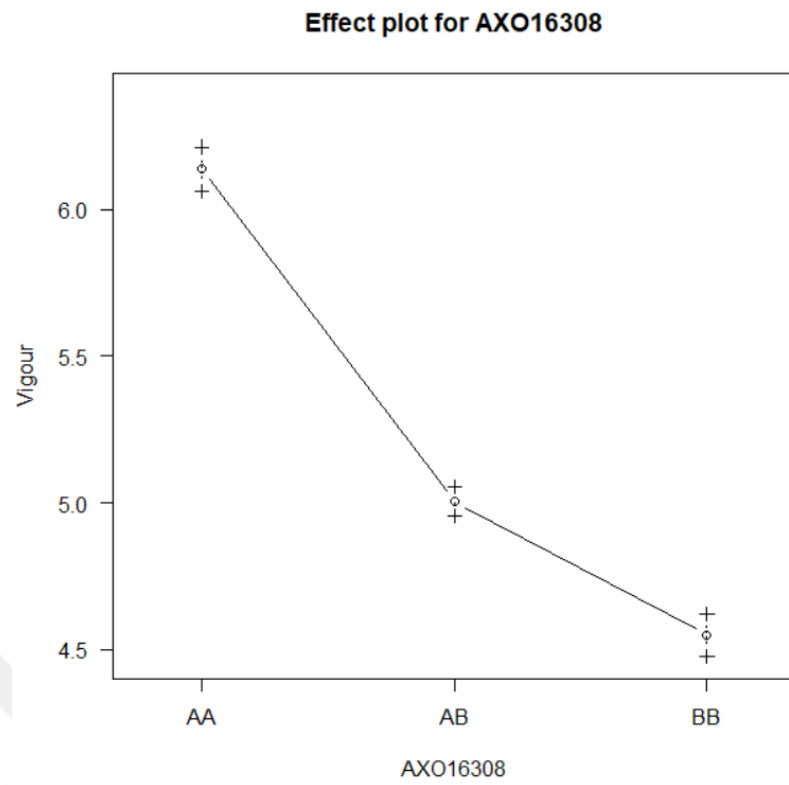


Figure 4.31. Vigour: Effect plot for the major QTL Vig.2.1.

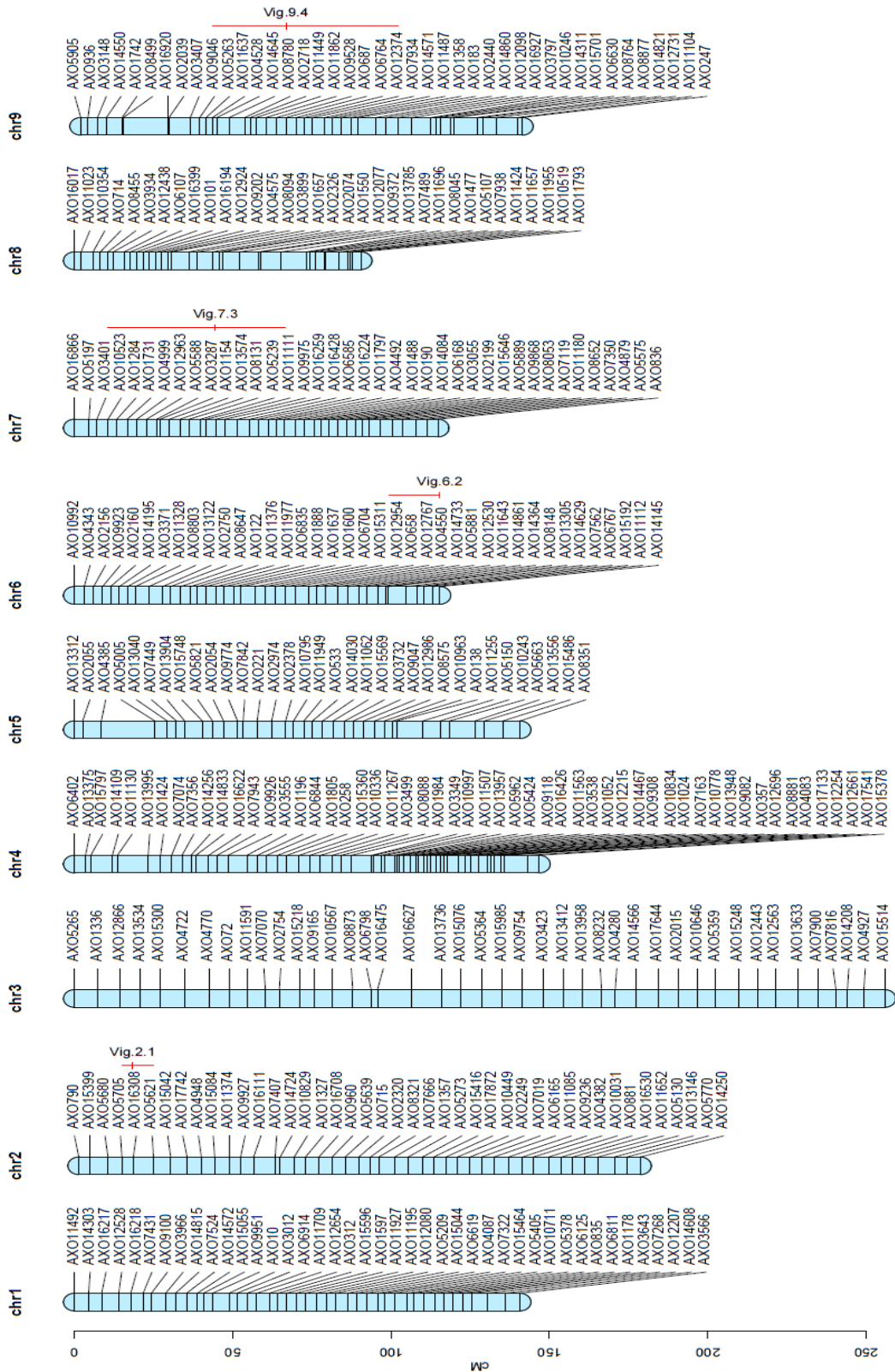


Figure 4.32. Cabbage Linkage map with marked QTLs for Vigour.

5. DISCUSSION

The purpose of this thesis research project was mapping QTLs for 12 important agronomic traits on early segment of white cabbage. The importance of this project is in a development of the markers linked to important agronomic traits and its application in cabbage breeding strategies. Marker assisted selection enables us to speed up the process of selection and elimination of potential white cabbage varieties.

The 61 putative QTL regions were found with seven QTLs being major QTLs. The most QTLs (19 of them) were located on a chromosome 2. It was surprising that six out of seven major QTLs were located in the same interval (14.99 cM – 25.01 cM) and linked to the same marker (AXO16308). Homozygous AA for the AXO16308 marker has shown higher trait values in cases of Stem length, Head length, Head shape, Colour, Anthocyanin and Vigour, while in case of Head density it showed decreasing values. (For example: an individual with homozygous AA for AXO16308 marker, had Head length of around 13cm, while homozygous BB individual had Head length of around 10 cm). This marker could be a powerful tool in further varietal selection and improvement, since manipulating only one marker could result in change of several agronomic traits.

In this research, QTLs for Leaf length were located on chromosomes 2, 3, 4, 5, 7, 8 and 9 and QTLs for Leaf width were located on chromosomes 2, 4, 7, 8, and 9. However there were no major QTLs and main regions for leaf length and width. Lv et al. (2016) examined 24 main agronomic traits on a DH mapping population of cabbage. They have also found that leaf - related traits were mainly located on chromosomes 2, 3 and 9, and two major regions including 37.3 – 39.9cM and 88.0 – 90.8cM on chromosome 3 which were for Leaf length and Leaf width (Lv et al., 2016). In this study, no QTLs were found for Leaf width on chromosome 3.

We found five QTLs for Head shape on chromosomes 2, 3, 5 and 6 and a major region located at 14.99 - 25.01 cM on chromosome 2. Lv et al. (2016) have also determined that important head – related QTLs, such as Head shape, were located on chromosomes 1, 2, 3, 5, 7 and 9, with major QTL regions at 59.0 - 60.9cM on chromosome 1, 12.6 – 27.2cM on chromosome 2, 88.0 – 90.8 cM on chromosome 3, 63.3 – 68.0cM on chromosome 5 and 58.6 – 61.5cM on chromosome 9 (Lv et al., 2016). While we did not determine any QTLs for Head shape on chromosome 1, 7 and 9 as earlier determined by Lv et al. (2016), there were QTLs on chromosome 6 which were not determined in earlier researches. The QTL region for Head shape on chromosome 2 was similar to the QTL region of Lv et al. (2016).

In this study, six QTLs for Head weight were determined on chromosomes 1, 2, 3, 5 and 8. In earlier research on Chinese cabbage, it was found that two QTLs for Head weight were located at 10.00 cM on chromosome 8 and at 62.56 cM on chromosome 6 (Sun et al., 2018). We determined that four QTLs for Head width were located on chromosomes 2, 3 and 5, and four QTLs for Head length were located on chromosomes 2, 6 and 7 with major QTL region being between at 14.99 and 25.01 cM. Su et al. (2018) found that two QTLs for Head length were located at 32.085 cM on chromosome 6 and at 16.07 cM on chromosome 3, and one QTL for Head width was located at 60.708 cM on chromosome 5. It can be stated that the differences of the QTLs for head related traits (Table 5.1) might be result from the use of different mapping population (DH versus F2 population), also from differences in parental materials (white cabbage versus Chinese cabbage).

Recently, the main focus in QTL research in cabbage and other Brassica crops has been mainly on *X.campestris* pv. *campestris* resistance (Iglesias-Bernabé et al., 2019), (Lee et al., 2015), (Fang et al., 2005), abiotic stress (Branham et al., 2016), (Matschegewski et al., 2015) and mineral composition (Wu et al., 2008).

Table 5.1. Comparison of thesis results and previous research

thesis results				previous research			
Trait	QTL	chromosome	position (cM)	QTL	chromosome	position (cM)	
Outer Leaf length	LfLen.2.1	2	130.36	Ll1.1	1	56.9–57	Lv et al. (2016)
	LfLen.3.2	3	145.5	Ll1.2	1	60.8–60.9	
	LfLen.4.3	4	33.5	Ll1.2	1	60.8–60.9	
	LfLen.5.4	5	55	Ll3.1	3	37.3–39.9	
	LfLen.7.5	7	25.95	Ll3.1	3	37.3–39.9	
	LfLen.8.6	8	5.88	Ll3.2	3	88–90.8	
	LfLen.9.7	9	72.69	Ll3.2	3	88–90.8	
Outer Leaf width	LfWid.2.1	2	15.86	Lw1.1	1	57.7–58.9	
	LfWid.2.2	2	128.61	Lw1.2	1	60.8–60.9	
	LfWid.4.3	4	12.02	Lw2.1	2	73.3–76.8	
	LfWid.7.4	7	25.95	Lw3.1	3	29.6–31.4	
	LfWid.8.5	8	5.88	Lw3.2	3	37.3–39.9	
	LfWid.9.6	9	62.47				
Head weight	HdWei.1.1	1	101	H_F2HWe1A8	8	10	Sun et al. (2018)
	HdWei.2.2	2	67.36	H_F2HWe2A6	6	62.563	
	HdWei.2.3	2	22.36				
	HdWei.3.4	3	139.5				
	HdWei.5.5	5	100.57				
	HdWei.8.6	8	4.5				
Head width	HdWid.2.1	2	26.86	H_F2HW1A5	5	60.708	
	HdWid.2.2	2	163.9				
	HdWid.3.3	3	139.5				
	HdWid.5.4	5	106.5				
Head length	HdLen.2.1	2	18.61	H_F2HH1A6	6	32.085	Lv et al. (2016)
	HdLen.2.2	2	95.86	H_F2HH2A3	3	16.071	
	HdLen.6.3	6	113.2				
	HdLen.7.4	7	25.95				
Head shape (HH/HW ratio)	HdRat.2.1	2	19.36	Hsi2.1	2	12.6–27.2	
	HdRat.3.2	3	61	Hsi4.1	4	72.8	
	HdRat.3.3	3	150	Hsi5.1	5	67.9–68	
	HdRat.5.4	5	132.5	Hsi5.2	5	77.4–78.8	
	HdRat.6.5	6	108.15	Hsi7.1	7	27.8–30.9	
				Hsi8.1	8	25.1	
				Hsi9.1	9	58.6–61.5	

6. CONCLUSION

Nine hundred and ten (910) plants of the early white cabbage F2 mapping population were grown and measured in Syngenta experimental station, in Hungary, during the period between March and June 2020. 369 polymorphic SNP markers were used to build the cabbage Linkage map which contains nine linkage groups (chromosomes). Thirteen phenotypic traits were evaluated. Twelve traits were used in QTL analyses in R/qtl program. Altogether, 61 putative QTL regions were found and among them 7 major QTLs (Table 6.1). Out of these seven major QTLs, six were found within the same interval (14.99 cM – 25.01 cM) and on the same chromosome (chromosome 2) (Figure 6.1). The marker closest to the StLen.2.2 (Stem length), HdLen.2.1 (Head length), HdRat.2.1 (Head shape), HdDen.2.2 (Head Density), Col.2.1 (Colour) and Vig.2.1 (Vigour) was AXO16308, while marker closest to the Ant.7.5 (Anthocyanin) was AXO14084. Epistasis was found in case of Head shape (HH/HW ratio).

Always growing need for yield improvement and crop adaptation to changing climate creates greater need for more thorough knowledge of agronomically important traits which define cabbage species; therefore, making this project as a good start material for further research. Further development of doubled haploid mapping populations and fine mapping with purpose of narrowing down the QTL positions and intervals would be recommended for future endeavours in this topic. Experimenting with mapping populations derived from different parental materials, grown in different regions and comparing it with existing results would give valuable insight that could enhance our chances for varietal improvement and development.

Table 6.1. Complete list of all QTLs found; Major QTLs are in bold

Trait name	QTL	Chromosome	position in cM	interval in cM	LOD	%var
Stem length	StLen.1.1	1	107.33	67.04 - 119.51	3.13	1.09
	StLen.2.2	2	17.86	14.99 - 25.01	48.02	19.05
	StLen.3.3	3	138.86	65.01 - 149.74	9.9	3.5
	StLen.6.4	6	111.5	104.84 - 115.38	19.32	7.01
	StLen.9.5	9	84.07	48.85 - 95.22	4.91	1.72
Outer Leaf length	LfLen.2.1	2	130.36	101.11 - 141.45	9.35	4.12
	LfLen.3.2	3	145.5	54.6 - 166.47	10.3	4.55
	LfLen.4.3	4	33.5	13.61 - 38.38	4.78	2.08
	LfLen.5.4	5	55	43.56 - 126.46	4.3	1.87
	LfLen.7.5	7	25.95	19.71 - 39.92	8.75	3.75
	LfLen.8.6	8	5.88	2.09 - 38.83	8.62	3.8
	LfLen.9.7	9	72.69	57.38 - 87.37	10.29	4.55
Outer Leaf width	LfWid.2.1	2	15.86	5.03 - 25.01	6.99	3.27
	LfWid.2.2	2	128.61	48.87 - 178.67	5.18	2.42
	LfWid.4.3	4	12.02	5.24 - 23.26	5.23	2.44
	LfWid.7.4	7	25.95	4.63 - 39.92	10.02	4.74
	LfWid.8.5	8	5.88	0.00 - 19.71	7.36	3.45
	LfWid.9.6	9	62.47	45.04 - 89.61	8.55	4.02
Leaf erectness	LfEre.1.1	1	93.5	24.33 - 107.33	6.69	2.99
	LfEre.2.2	2	21.86	14.99 - 30.35	14.13	6.5
	LfEre.3.3	3	51	42.48 - 60.29	11.95	5.42
	LfEre.7.4	7	37.5	0.00 - 55.44	5.9	2.63
Head weight	HdWei.1.1	1	101	79.66 - 122.84	6.69	2.66
	HdWei.2.2	2	67.36	56.64 - 81.37	21.5	8.91
	HdWei.2.3	2	22.36	18.61 - 30.35	14.06	5.07
	HdWei.3.4	3	139.5	122.02 - 196.82	9.9	4
	HdWei.5.5	5	100.57	78.36 - 110.04	9.5	3.79
	HdWei.8.6	8	4.5	0.00 - 29.04	4.45	1.8
Head width	HdWid.2.1	2	26.86	18.61 - 35.60	7.7	4.4
	HdWid.2.2	2	163.9	113.49 - 170.49	10.4	6.04
	HdWid.3.3	3	139.5	122.02 - 154.54	10.8	6.28
	HdWid.5.4	5	106.5	78.36 - 115.52	4.55	2.6
Head length	HdLen.2.1	2	18.61	14.99 - 25.01	32.73	17.1
	HdLen.2.2	2	95.86	56.64 - 137.06	19.24	9.6
	HdLen.6.3	6	113.2	95.99 - 115.38	3.58	1.69
	HdLen.7.4	7	25.95	0.00 - 51.58	7.47	3.58
Head shape (HH/HW ratio)	HdRat.2.1	2	19.36	14.99 - 25.01	76.07	37.09
	HdRat.3.2	3	61	34.75 - 106.41	6.45	2.46
	HdRat.3.3	3	150	135.22 - 196.82	8.53	3.27
	HdRat.5.4	5	132.5	97.87 - 140.71	4.63	1.75
	HdRat.6.5	6	108.15	92.39 - 115.38	4.95	1.88

Table 6.1. Complete list of all QTLs found; Major QTLs are in bold (continued)

Trait name	QTL	Chromosome	position in cM	interval in cM	LOD	%var
Head density	HdDen.1.1	1	78.5	70.01 - 125.57	12.07	5.28
	HdDen.2.2	2	18.86	14.99 - 25.01	44.29	21.67
	HdDen.2.3	2	117.26	63.39 - 153.89	5.57	2.38
	HdDen.4.4	4	13.61	5.24 - 84.39	4.57	1.95
	HdDen.4.5	4	146.79	33.96 - 146.79	4.3	1.81
	HdDen.5.6	5	102.5	97.87 - 115.52	4.77	2.04
	HdDen.6.7	6	44.74	0.00 - 76.65	5.24	2.24
Colour	Col.2.1	2	20.86	14.99 - 25.01	33.87	13.72
	Col.2.2	2	86.86	65.02 - 161.07	12.01	4.58
	Col.3.3	3	122.02	54.61 - 128.81	5.54	2.08
	Col.7.4	7	41.67	30.01 - 51.58	8.27	3.12
Anthocyanin	Ant.1.1	1	50.5	43.64 - 73.03	7.22	3.03
	Ant.2.2	2	16.86	5.03 - 25.01	9.74	4.13
	Ant.2.3	2	68.36	52.48 - 85.47	10.4	4.4
	Ant.4.4	4	3.45	0.00 - 36.82	4.43	1.85
	Ant.7.5	7	74	69.65 - 75.88	33.29	15.07
Vigour	Vig.2.1	2	18.36	14.99 - 25.01	48.25	20.87
	Vig.6.2	6	115.38	99.24 - 115.38	6.56	2.53
	Vig.7.3	7	44.5	10.57 - 66.43	3.67	1.4
	Vig.9.4	9	66.78	43.5 - 102.18	6.15	2.37

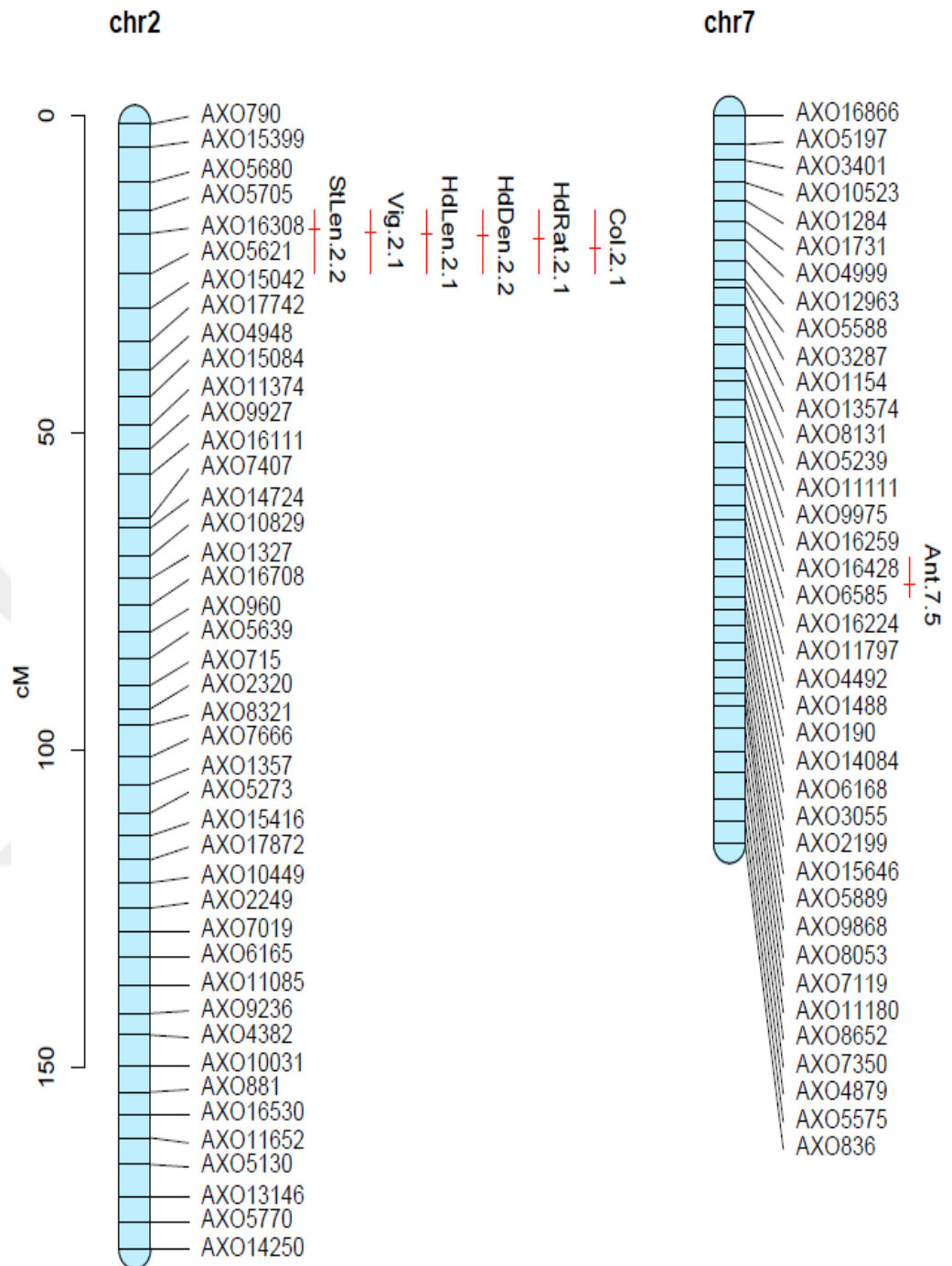


Figure 6.1. Position of the seven Major QTLs on the Linkage map.

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25/09/2020

A handwritten signature in blue ink, reading 'Katarina Jović', with a stylized flourish at the end.

Katarina JOVIĆ

CURRICULUM VITAE

Katarina Jović was born on 29th September 1995. in Bijeljina, Bosnia and Herzegovina. She has completed her Bachelor studies in 2017, on Szent Istvan University in Godollo, Hungary, as one of the Stipendium Hungaricum scholars, with the average grade of 4.81 (highest grade being 5.0). She was admitted to Erasmus Mundus Master program in Plant Breeding (emPlant) at UniLaSalle in Beauvais, France, where she has completed her first year of Master program, in 2019. She started her second year of Master program at Ege University, in Izmir, Turkey. She did her 6 months thesis internship at Syngenta experimental research station, in Ocsa, Hungary, in 2020.

