IDENTIFICATION OF GENOMIC REGIONS CONTROLLING EARLY PLANT VIGOUR IN LENTIL (*Lens culinaris* Medik.)

THESIS

By

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(A-2016-30-005)

Submitted to

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CERTIFICATE – I

This is to certify that the thesis entitled **"Identification of genomic regions controlling early plant vigour in lentil (*Lens culinaris* Medik.)"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Agricultural Biotechnology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Mr. Rushikesh Mane (A-2016-30-005)** son of **Shri. Sanjay Mane** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

(Dr. R.K. Chahota)
Major Advisor

Place: Palampur
Dated: 7th January, 2019

CERTIFICATE- II

This is to certify that the thesis entitled**, "Identification of genomic regions controlling early plant vigour in lentil (*Lens culinaris* Medik.)"** submitted by **Mr. Rushikesh Mane (A-2016-30-005)** son of **Shri. Sanjay Mane** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Agricultural Biotechnology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Place : Palampur
Dated : 7th January, 2019 ***(Rushikesh Mane)***

TABLE OF CONTENTS

LIST OF ABBREVIATIONS USED

Sr. No.	Abbreviation	Meaning
	%	Percent
	µg	Microgram
	µl	Microlitre
	AFLP	Amplified Fragment Length Polymorphism
	Bp	base pair
	CAPS	Cleaved Amplified Polymorphic Sequence
	cM	centi Morgan
	CTAB	Cetyl Trimethyl Ammonium Bromide
	dATP	Deoxyadenosine triphosphate
	dCTP	Deoxycytosine triphosphate
	dGTP	Deoxyguanosine triphosphate
	DNA	Deoxyribonucleic Acid
	DTS	Drought Tolerance Score
	DTF	Days to Flower
	dNTP	Deoxynucleotide triphosphate
	DAS	Days after Sowing
	dTTP	Deoxythymidine triphosphate
	EDTA	Ethylenediamine Tetra Acetic Acid
	EST-SSR	Expressed Sequence Tagged-Simple Sequence Repeats
	et al	And Coworkers
	Fig	Figure(s)
	G	Gram
	g SSR	Genomic Simple Sequence Repeats
	GLLC	Grain Legume *Lens culinaris*
	H	Hour
	HCl	Hydrochloric acid
	ISSR	Inter Simple Sequence Repeats
	ITAP	Intron-Targeted Amplified Polymorphic Sequence
	Kb	Kilobase
	RCS	Red clover Simple Sequence Repeat
	LcSSR	*Lens culinaris* Simple Sequence Repeat
	LG	Linkage Group
	LOD	Log of Odds
	M	Molar
	Mbp	Million base pairs

Mg	Milligram
$MgCl_2$	Magnesium Chloride
Min	Minute(s)
Ml	Millilitre
mM	Millimolar
NaCl	Sodium Chloride
Ng	Nanogram
^oC	Degree Celsius
P	Probability
PCR	Polymerase Chain Reaction
PH	Plant height
pH	Puissance de hydrogen (ion conc.)
ppm	Parts per million
PVP	Polyvinylpyrrolidone
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RIL(s)	Recombinant Inbred Line(s)
RNase	Ribonuclease
Rpm	Revolutions per minute
Sec	Second(s)
SNPs	Single Nucleotide Polymorphisms
SRAP	Sequence Related Amplified Polymorphism
SSR	Simple Sequence Repeat(s)
STMS	Sequence Tagged Micro Satellite(s)
STS	Sequence Tagged Site
Taq polymerase	*Thermusaquaticus* DNA polymerase
TE	Tris EDTA buffer
Tris	Tris (hydroxy methyl) amino methane
U	Units
UV	Ultraviolet
V	Volts

LIST OF TABLES

LIST OF FIGURES

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Abstract

The present study was aimed at development of PCR-based linkage map of an F_{10}RIL population of 94 individuals (derived from WA8649090 x Precoz) of lentil and identification of genomic regions linked to early plant vigour traits. Of the 268 (93 SSRs, 3 ISSRs and 172 RAPDs) markers, 265 (90 SSRs, 3 ISSRs and 172 RAPDs) were mapped on seven linkage groups, varying in length between 25.6 to 210.3 cM and covering 809.4 cM with an average marker spacing of 3.05 cM. Number of markers per linkage group varied from 8-76. Seventy one distorted markers were mapped in the linkage map. The study also reported assigning of 24 new cross- genera SSRs of *Trifolium pratense* on the present linkage map.

Analysis of variance showed considerable genetic variation for all the traits in parental lines and RILs. Shoot length, Root length, Seedling length, No. of leaves and No. of branches showed normal frequency distribution curve whereas Dry weight showed skewed Distribution curve. A compact genomic region (QTL HOTSPOT) consisting of 6 QTLs for Shoot length (cm), Root length (cm) and Seedling length (cm) was observed within a map distance of 56.61 to 86.81 cM on LG1. Some linkage groups showed lack of association with quantitative traits, leading to uneven distribution of QTLs across genome.

The QTLs identified for the traits studied have implications for accelerating the process of pyramiding of favourable genes into adapted genotypes through marker-assisted breeding in lentil. The knowledge of marker-trait association may lead to the identification of genes influencing early plant vigour traits in lentil. Therefore, the PCR- based linkage map and QTL mapping is an effort to provide a basis for MAS in future lentil breeding programs.

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1. INTRODUCTION

Lentil (*Lens culinaris* Medikus) is one of the early domesticated plant species, as old as those of einkorn, emmer, barley and pea (Harlan 1992). In India, lentil is grown as a winter crop, mainly in Bihar, West Bengal, Madhya Pradesh, Uttar Pradesh, Haryana, Punjab, Maharashtra and parts of Himachal Pradesh, especially in low and mid hills in an area of 1.50 m ha with annual production of 1.05 m tones (FAOSTAT 2016).It is an ancient crop that is believed to be originated in the near East and later spread all through the Mediterranean Basin and central Asia (Cubero et al. 2009). The plant was given the scientific name *Lens culinaris* in 1787 by Medikus, a German botanist and physician (Cubero 1981; Sehirali 1988; Hanelt 2001).

Lentil belongs to the Kingdom *Plantae*-Plants; Subkingdom *Tracheobionta*-Vascular plants; Super division *Spermatophyta*-Seed plants; Division *Magnoliophyta*-Flowering plants; Class *Magnoliopsida*-Dicotyledons; Subclass *Rosidae*; Order *Fabales*, Family *Fabaceae*-Pea family; Genus *Lens* Mill.-lentil; and Species *Lens culinaris* Medik.- lentil (Anonymous 2012). Lentil (*L. culinaris* ssp. *culinaris*), is a diploid (2n=2X=14) self-pollinated with a genome size of approximately 4 Gbp (Arumuganathan and Earle 1991).The genus Lens consists of four species, 3 of which are wild (*Lens ervoides; Lens lamottei and Lens nigricans*) (Van Oss et al. 1997) and *Lens culinaris* Medikus which consists of both cultivated and wild subspecies. The wild relatives serve as a reservoir of genetic variability and agronomical desirable traits which could be utilized in molecular breeding programs for genetic improvement of lentils (Sarker et al. 2006). Lentil is an important source of dietary protein in both the human diet and in animal feed, and it also helps in the management of soil fertility for higher yield.With about 28% of protein content, lentil seeds are considered to be a rich source of protein for human consumption and its straw is used as feed for animal as it is high in mineral and carbohydrate content (Muehlbauer et al. 2006). India ranks second in Lens production after Canada whereas the world's largest exporters are Canada and Turkey (FAOSTAT 2012). It is an important cool

season crop grown widely throughout the Indian subcontinent, northern Africa, western Asia, southern Europe, North and south America and Australia. The cultivated variety *L. culinaris* spp. *culinaris* encompasses two forms of cultivated lentils on the basis of physio-morphological traits, the small seeded (*microsperma*) and large-seeded (*macrosperma*). It provides affordable source of dietary proteins (22-35%), minerals, fiber, and carbohydrates to poor people and micronutrient deficiencies in developing countries. As it exhibits low glycemic index, it is highly recommended by physicians for the people suffering from diabetes, obesity, and cardiovascular diseases (Srivastava and Vasishtha, 2012). In fact, vegetable protein is gaining preference over the animal protein for consumption by the health-conscious people in the present day. This could be one of the reason for increased per capita consumption (Vandenberge, 2009) and five-fold increase in global lentil production (from 0.85 to 4.43 mt) during the last five decades, through a 155% increase in sown area and the doubling of average yields from 528 to 1068 kg ha^{-1}(Faostat, 2015). Lentil cultivation often provides rotational benefits to cereal-based cropping systems through biological nitrogen fixation, carbon sequestration, and through effective control of weeds, disease and insect pests. It generates livelihood for the small-scale farmers practicing agriculture in the dryland agricultural ecosystems of South Africa, Sub-Saharan Africa, West Asia, and North Africa (Kumar et al., 2013). In India, lentil occupies about 1.48 m ha with annual production of 1.10m tones.

The availability of molecular maps has facilitated gene tagging, marker-assisted selection and the positional cloning of resistance genes. The first genetic maps of lentil consisted of a small number of markers, mainly of isozymes, restriction fragment length polymorphisms (RFLPs) and some morphological markers that covered a relatively small portion of the genome (Havey and Muehlbauer 1989; Weeden et al. 1992; Tahir et al. 1993). The *Lens* sp. map constructed by Eujayl et al. (1998a) was based on 86 recombinant inbred lines (RILs) and consisted primarily of 89 random amplified polymorphic DNAs (RAPDs) and 79 AFLPs together with six codominant markers, most of the latter being RFLPs. The lentil linkage map developed by Rubeena et al. (2003) using an F_2 population encompassed this was followed of 100 RAPDs, 11 ISSRs (inter-simple sequence repeats) and three resistance gene analog (RGA) markers. *Lens* sp. map of 62 RAPDs, 29 ISSRs,

65AFLPs and four morphological and one microsatellite marker (Dura´n et al. 2004). Microsatellites or simple sequence repeats (SSRs) are small tandem repeats of DNA, usually 2–5 bp in length, that occur in most eukaryotic genomes. Microsatellites or simple sequence repeat (SSR) markers are the markers of choice because of locus specificity, hyper variability, co-dominance and higher producibility (Powell et al. 1996; Varshney et al. 2005; Varshney et al. 2009) and have proved to be promising for various genomic applications (Gupta et al. 2000). They are being widely applied in plant genome mapping and phylogenetic analysis because of their co-dominant inheritance and high degree of polymorphism. They arise due to unequal crossing over or replication slippage and represent the hyper variable regions of the genome which differ because of repeat motif number variation (Schlotterer et al. 1992). However, the flanking regions of the microsatellites are conserved within individuals of a given species and are thus used for designing locus specific microsatellite markers. Microsatellite markers had been used in various plants for genome mapping (Roder et al. 1998) and tagging various traits of agronomic importance.Although lentil is an economically important legume, its genetic and genomic resources remain largely uncharacterized and unexploited. However, during the past few years efforts have been made towards developing DNA based molecular markers to directly link the trait of interest for the development of improved crop varieties.These resources could be exploited for understanding the extent and distribution of genetic variation available within the genus *Lens* and for developing saturated genetic maps suitable for QTL mapping and marker-assisted selection.

With the development of DNA markers and the construction of high-density linkage maps for many plant species, the QTL (quantitative trait loci) mapping technique has become a powerful tool for identifying genomic regions affecting quantitative traits by providing information on the map location, relative effect, gene action and dominance properties of each identified locus (Lander and Botstein 1989; Tanksley 1993). Recently, a few QTL for seedling vigour in rice have been identified by the QTL mapping approach, and some of these are expected to be useful for the improvement of seedling vigour by pyramiding the positive alleles via marker-assisted selection (MAS) (Redona and Mackill, 1996b; Cui et al. 2002; Fujino et al. 2004). The significance of genotype environment (GE) interactions in the genetic

control of quantitative traits has long been recognized by quantitative geneticists (Moll et al. 1978). Because of the GE interaction, QTL that are important in one environment may not be as important as in another environment in determining the phenotype (Tanksley 1993). For this reason, QTL with little GE interaction across a set of environments would be desirable in marker-assisted breeding programs. With rapid advancement of molecular technology, it is now possible to use molecular marker information to map major QTLs on chromosomes (Paterson et al. 1988). QTL mapping provides a means to dissect complex phenotypic characters into their component traits (QTLs) and allows the identification of molecular markers linked to desirable QTLs, so that these can be directly used in marker-assisted selection (Tanksleyet al.1989; Lee 1995; Schneider et al.1997; Mohan et al.1997). Linkage maps are useful for locating quantitative traits and understanding the genetic make-up of a crop. Genetic linkage maps in lentil have been constructed (Zamir and Ladizinsky 1984; Tadmor et al. 1987; Havey and Muehlbauer 1989; Tahir et al. 1993; Eujayl et al. 1997; Duran et al. 2004; Gupta et al. 2012b; Fedurok et al. 2013; Verma et al., 2015).Thus, the purpose of this study was to ascertain the genomic position, number and magnitude of QTLs affecting genetic variation for a number of physiological, biochemical, morphological, phenotypical and yield-related traits in RIL populations derived from a cross between two lentil genotypes showing contrasting expression for plant early vigour traits. The study thus provides valuable information on the feasibility of using QTLs in a marker-assisted selection scheme to identifying genomic regions controlling plant early vigour traits. Identification of QTLs for important agronomic traits has been made possible in a number of plant species with the availability of polymorphic markers and linkage maps. Plant early vigour traits, like seedling length, root length, shoot length and dry weight are economically important quantitative traits, which are believed to be controlled by multiple genes. Germination rate and early seedling growth are the major seedling-vigor related traits. Rapid shoot and root growth were observed to be closely associated with seedling-vigor (Williams and Peterson 1973). The early vigour traits are important for determining an establishment in the field as early as possible. It is generally considered that carbohydrates for early seedling growth before seedlings gain the ability of photo autotrophy are provided by breakdown of the starch stored in the

endosperm. Thus, functionally, it should be expected that amylase activities are correlated with germination rate and early seedling growth in rice, as was reported previously (Williams and Peterson 1973). The application of molecular-marker technology and QTL mapping has facilitated understanding of the genetic basis of many agriculturally important quantitative traits and phenomena. However, QTL analyses have largely been used for agronomic and morphological traits. Physiological traits are more closely related to gene expression than agronomic traits. In recent years, there are also increasing interest in mapping QTLs for physiological traits (Causse et al. 1995; Prioul et al. 1997; Mitchell-Olds and Pedersen 1998; Quarrie et al. 1999; Sanguineti et al. 1999). Plant early vigour traits are morphological traits and one of the important components of plant yield and the major target for breeding. The present productivity of lentil in India is very low as it is mostly grown as post-rainy season crop under receding soil moisture conditions during the winter season. As a result, the crop invariably encounters terminal moisture stress, thus, leading to forced maturity and lower yield. Early growth vigour and subsequent rapid ground coverage has been shown to be associated with drought tolerance. This trait is necessary to optimize the utilization of the production environment by reducing surface soil evaporation. Genetic variation in early growth vigour has been reported in many grain legumes (Onim 1983; Silim et al. 1993), which indicates the feasibility of manipulating this trait through classical breeding techniques. In lentil, genetic variation for faster growth rate was observed and a positive association has been observed between stem length and tap root length as well as number of lateral roots (Sarkeret al. 2005). These workers have also identified a breeding line (ILL 6002), which exhibited a faster growth rate and formed a large number of lateralat early stage of seedling establishment (Sarker et al. 2005).These studies showed that genetic variability of rapid early growth vigour is present among the lentil germplasm. To utilize this genetic variability in lentil breeding programme, it is essential to investigate the genetic control of early growth vigour. Knowledge of genetics of a trait helps to choose the parents and construct an appropriate breeding scheme (Sarker et al. 1999). Previously genetics of quantitative traits such as days to flowering and resistance to Ascochyta blight have been shown to be inherited in Mendelian fashion in lentil (Ford et al. 1999; Sarker et al. 1999). Seed vigor is defined as “seed

properties that determines potential for fast and uniform emergence and development of seedlings under a wide range of field conditions". Generally, low germination speed, high sensibility to stresses of seeds and seedlings during germination process and plants with slow, low and irregular growth or with less root development are typical characteristics of seed with low physiological potential. Seed vigor has a high influence on establishment of initial population of plants as well as on their adequate development, what will affect crop yield. This way, seeds have low vigour could diminish percentage of emerged seedlings and seedling emergence speed, initial growth, leaf area, and dry mass accumulation. High seedling vigour helps the genotypes to suppress the weeds, which is a serious problem in large rain fed and upland areas in the tropics where dry seeding is practiced. The purpose of this study was to identify quantitative trait loci (QTLs) underlying seedling vigour-related traits using a RIL mapping population derived from a cross between a *WA8649090* and Precoz.

Thus, the present study was undertaken to enrich a PCR-based linkage map and to identify markers linked to the genes controlling early plant vigour traits on an F_{10} RIL population.

In light of the above, the present investigation was carried out with the following objectives:

1. To enrich the lentil linkage map developed using intraspecific RIL mapping population
2. To identify QTLs linked to early plant vigour traits

2. REVIEW OF LITERATURE

The literature pertaining to different aspects of the present investigation has been reviewed under the following heads:

1. **Origin, Distribution, Taxonomy and Production of Lens**:

Cultivated lentil is thought to have been originated and first domesticated in western Asia and then introduced into the Indo-Gengetic plain around 2000 BC (Cubero 1981). Lentil has also been rapidly spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India, Pakistan, China and later to the new world including Latin America, Mexico, Chili, Argentina, Colombia and more recently Canada (Cokkizgin et al. 2013). According to Cubero (Cubero 1984) lentil was first spread to the Nile from the Near East, to Central Europe and then to the Indian Subcontinent and the Mediterranean Basin by the end of Bronze Age. It is now cultivated in most subtropical and also in Northern hemisphere such as Canada and Pacific Northwest regions (Oplinger et al. 1990). Lentil belongs to the genus *Lens* of the *Viceae* tribe in the *Legumnosae* (*Fabaceae*) family, commonly known as the legume family (Fikiru et al. 2007). The plant was given the scientific name *Lens culinaris* in 1787 by Medikus, a German botanist and physician (Hanelt P 2001). The cultivated lentil, Lens *culinaris* spp. *culinaris*, has two varietal types: small seeded (*microsperma*) and large seeded (*macrosperma*) (Sharma et al.1995). It is an annual bushy herb with slender stem and having many branches with erect, semi-erect or spreading growth habit (Sandhu et al. 2007). Lentil plants are typically short, but can range from 20 to 75cm in height, depending on growing conditions (Saskatchewan Pulse Growers 2000). The cotyledons remain under ground after germination. It is an annual cool-season self-pollinated diploid (2x = 2n = 14 chromosomes) and world's fifth largest pulse crop with an annual production of about 1.05 Mt (FAOSTAT 2014). Its wild progenitor (Lens culinaris ssp. orientalis (Boiss.) Ponert) has a wide distribution, but the genetic stock from which the crop originated was probably found in the Near East (Zohary 1972). It is supposed that this crop is the result of a single domestication event (Zohary 1999).Lentil plays a significant role in

human and animal nutrition and in maintenance and improvement of soil fertility (Sarker et al. 2011). Its cultivation enriches soil nutrient status by adding nitrogen, carbon and organic matter which promotes sustainable cereal-based systems of crop production (Sarker et al. 2011). It is a nutritious food legume. It is cultivated for its seed and mostly eaten as split (Iqbal et al. 2006). The primary product of lentil is its seed which has relatively higher contents of protein, carbohydrate and calories compared to other legumes (Muehlbauer et al. 1985). It is the most desired crop because of its high average protein content and fast cooking characteristic in many lentil producing regions. It can be used as a main dish, side dish, or in salads. Seeds can be fried and seasoned for consumption but sometimes difficult to cook because of the hard seed coat those results from excessive drying (Winch 2006). Its flour is used to make soups, stews, purees, and mixed with cereals to make bread and cakes; and as a food for infants (Williams et al. 1988).

The seeds are highly nutritious and contain almost all the essential nutrients for human consumption especially protein, carbohydrate, vitamins, micronutrients (K, P, Fe, Zn) and β-carotene (Erskine and Sarker 2004). Even its straw has sufficient elements and is used as an animal feed (Erskine et al. 1990). It is commonly called as *masoor* in India. Lentil being an important and popular legume, it has numerous other names which are used in different other countries such as *messer* (in Ethiopia), *heramame* (in Japan), *adas* (in Arabic) and *mercimek* (in Turkey). Because of high protein content and easy accessibility by the lower economic class it is referred to as "poor man's meat" by the developing and higher class. It has all the essential elements similar to other pulse crops including high folate content and high cholesterol-lowering soluble fiber and thus is a valuable source of functional food for humans. It also has high content of lysine and tryptophan content but lacks in sulfur containing amino acids like methionine. However, its consumption with cereal crops like wheat and rice fulfills the requirement of other essential amino acids which is lacking in lentil and thus provides a complete balance of diet. Like other legumes it also has the tendency to fix atmospheric nitrogen thus increases soil fertility, so it is usually grown in rotation with cereal crops, providing biological benefits to the cropping systems.

2. Development of linkage map using PCR-based markers

The analysis of DNA sequence variation is of major importance in genetic studies. In this context, molecular markers are a useful tool for assaying genetic variation, and have greatly enhanced the genetic analysis of crop plants. A variety of molecular markers, including restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites or simple sequence repeats (SSRs), have been developed in different crop plants (Philips et al. 2001; Varshney et al.2004). With the advantage of SSRs over other markers, SSRs were also developed in lentil. However only few reports have been published earlier, related to SSR development in lentil (Závodná et al. 2000; Hamwieh et al. 2005; Phan et al. 2007; Hamwieh et al. 2009; Saha et al. 2010b) and some of them have been utilized for map construction (Durán et al. 2004; Hamwieh et al. 2005; Hamwieh et al. 2009; Saha et al. 2010b). More recently a set of 122 and 360 new genomic SSR markers were reported by Verma et al. (2014) and Andeden et al. (2015), respectively. Further, a repertoire of 501 *Lens* SSR markers was developed using two microsatellite genomic libraries enriched for (GA/CT) and (GAA/CTT) motif (Verma et al. 2015).Among different classes of molecular markers, SSR markers are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genome coverage (Powell et al. 1996). SSR markers have been useful for integrating the genetic, physical and sequence-based physical maps in plant species, and simultaneously have provided breeders and geneticists with an efficient tool to link phenotypic and genotypic variation (Gupta et al. 2000). With the establishment of expressed sequence tag (EST) sequencing projects for gene discovery programs in several plant species, a wealth of DNA sequence information has been generated and deposited in online databases (Rudd 2003).Genetic linkage maps are valuable resources that provide a framework for many genomic analyses. They are an important tool for many genetic applications, including mapping of quantitative trait loci (QTLs), comparative mapping, identifying DNA markers for fingerprinting, analyses of population genetics and phylogenetic, genome sequence assembly, relating physical and recombination distances along the genome, and map-based cloning of genes. The wide applications of linkage maps and

their importance in genetic research led to numerous linkage mapping projects being undertaken in plants. Through the use of linkage maps, characterization of quantitatively inherited traits has been facilitated, including identifying the genomic regions containing contributing loci, postulating the types of gene action that may be involved and determining the role of epistatic effects in specifying phenotype (Tanksley 1993). In modern genetic analysis both the marker systems i.e. SSRs and SNPs have been found to be valuable for linkage mapping and QTL identification. Even though SNPs provide a number of advantages, SSRs are found to be more polymorphic and are considered as the best marker system for construction of framework linkage map (Jones et al. 2007). Therefore it became imperative to isolate microsatellites from lentil and utilize them to construct a framework linkage map to identify QTLs for important agronomic traits. Identification of QTLs for important agronomic traits has been made possible in a number of plant species with the availability of polymorphic markers and linkage maps. As morphological and isozyme markers are generally unsuitable, the introduction of molecular markers was critical. Though morphological and isozyme markers were used to identify the first genetic linkages in lentil (Muehlbauer et al. 1989; Tadmor et al. 1987; Vaillancourt and Slinkard 1993; Zamir & Ladizinsky 1984). Soon thereafter many types of DNA-based molecular markers, arising from point mutations, insertions or deletions or errors in replications of tandem-repeated DNA, were identified and used for mapping the lentil genome. Restriction fragment length polymorphism (RFLP) markers, developed from cutting genomic DNA with restriction enzymes and separating the resulting DNA fragments with electrophoresis, were the first type of molecular marker used in the construction of a lentil genetic linkage map (Havey & Muehlbauer 1989). Subsequently, arbitrarily produced polymerase chain reaction (PCR)- based markers, such as random amplified polymorphic DNA (RAPD) were used to study diversity, phylogeny and taxonomy of *Lens* (Ford et al. 1997; Ferguson et al. 2000; Sharma et al. 1996), to develop linkage maps (Eujayl et al. 1997, 1998a; Rubeena et al. 2003), to tag genes of interest (Chowdhury et al. 2001; Eujayl et al. 1998; Ford et al. 1999; Tullu et al. 2003) and to determine pathogen population structure (Ford et al. 2000). Arbitrarily produced amplified fragment length polymorphism (AFLP) markers have also been used in lentil linkage mapping (Duran et al. 2004; Eujayl et al.

1998a; Hamwieh et al. 2005; Kahraman et al. 2004) and to study genetic diversity (Sharma et al. 1996), differentiate cultivars (Z´avodn´a et al. 2000) and identify markers linked to specific traits (Tullu et al. 2003). Comparisons of the lentil linkage map with that of chickpea and pea indicate interesting similarities. More recently, simple sequence repeat (SSR) or microsatellite markers, which consist of tandem repeats of two to five nucleotide DNA core sequences that are spread throughout the genome, were used to construct lentil linkage maps (Dur´an et al. 2004; Hamwieh et al. 2005). The DNA sequences flanking microsatellite repeats are generally conserved within individuals of a given species, allowing the design of highly specific and robust PCR primers that amplify the intervening SSR. Two sets of microsatellite markers were developed from lentil by Z´avodn´a et al. (2000) and at ICARDA by Hamwieh et al. (2005). The ICARDA SSR library was developed from the genome of the Northfield cultivar (ILL5588) and was found to have (CA)n as the most abundant repeat type (Hamwieh et al. 2004). Other marker types such as inter-simple sequence repeat (ISSR) markers, amplified with SSR-anchored primers, and resistance gene analogue (RGA) markers, amplified with degenerate primers designed from conserved regions of cloned plant resistance genes were also used in lentil genome mapping (Dur´an et al. 2004; Rubeena et al. 2003). Positional cloning ofRGAmarkers will be potentially aid in the localization of disease resistance genes via the candidate-gene approach (Kanazin et al. 1996; Leister et al. 1996). Early pioneering studies suggested a simple genetic relationship between pea and lentil (Weeden et al. 1992) and within grasses and crucifers shortly thereafter. Subsequent comparative genetic studies of increasing sophistication have demonstrated diVering degrees of synteny between chickpea (*Cicer arietinum*) and pea (*Pisum sativum*, Simon and Muehlbauer 1997), mung bean (*Vigna radiata*) and cowpea (*V. unguiculate*, Menancio- Hautea et al. 1993), mung bean and lablab (*Lablab purpureus*, Humphry et al. 2002), and alfalfa (*Medicago sativa*) and pea. Building on these foundations has required the generation of abundant genomic and genetic resources focused around model species. In the case of legumes, the two models are *M. truncatula* and *L. japonicus.* Early work tended to use RFLP probes based on the genome sequence of model species. Although RFLP probes are more polymorphic and better at detecting duplications, but they are laborious and have therefore been superseded by PCR techniques. PCR-based,

codominant marker systems for comparative genomics have markedly increased the efficiency of transferring genetic information across species. In this approach, oligonucleotide primers are designed from sequences of conserved regions, for example in gene exons that span polymorphic regions such as introns or microsatellites. Examples include the comparison of *Medicago truncatula* with alfalfa, pea, chickpea and lupins (Aubert et al. 2006; Choi et al. 2004; Nelson et al. 2006) reviewed in Zhu et al. (2005). A detailed linkage map is required to define and distinguish QTL. Once major QTLs have been uncovered, tightly linked markers may be validated for use in marker-assisted selection (MAS) and potentially even as a starting point for the positional cloning of the underlying functional resistance gene(s) (Haley and Anderson 1997).

An extensive linkage map of *Lens* with 86 RILs comprising 177 markers (89 RAPD, 79 AFLP, six RFLP and three morphological markers) that covered 1,073 cM with an average marker distance of 6.0 cM was developed by Eujayl et al. (1998). The large proportion of 37 unlinked markers reflects the need for screening a large number of markers to evenly cover the lentil genome. They observed only 8.4% marker distortion, most probably due to previous selection against segregation distortion at the F_2 stage (Eujayl et al. 1997). However, in another cross, a segregation distortion of 83.3% was reported (Eujayl et al. 1997). In the lentil map, distorted loci were observed at the end or middle of linkage groups.

The lentil linkage map developed by Rubeena et al. (2003) using an F_2 population encompassed 100 RAPD, 11 SSR and 3 RGA markers. Of the 118 markers screened, 114 (96.6%) were mapped and four (two RAPD and two ISSR) remained unlinked. The analysis revealed nine linkage groups varying in length from 34.9 cM to 134.8 cM. The map spanned a total length of 784.1 cM with an average marker density of 6.9 cM between adjacent markers. The intersubspecific F_2 lentil genetic map produced by Durán et al. (2004) at LOD score of 3.0 comprised of 161 markers (62 RAPD, 29 ISSR, 65 AFLP, four morphological markers and one SSR) with ten linkage groups. The markers spanned a distance of 2172.4cM with an average distance between markers of 15.87 cM. The lentil linkage map of Kahraman et al. (2004) covered 1192 cM within nine linkage groups and comprised a total of 130 RAPD, ISSR, and AFLP markers. Thereafter, SSR linkage map of lentil was

constructed by Hamwieh et al. (2005). They developed a lentil map with 39 new lentil-specific SSRs and 50 new AFLP markers. The map comprised of 283 markers spanning 751 cM with an average marker distance of 2.6 cM within 14 linkage groups for localization of Fusarium vascular wilt resistance gene. The DNA sequences flanking microsatellite repeats are generally conserved within individuals of a given species, allowing the design of highly specific and robust PCR primers that amplify the intervening SSR. The use of SSR markers in lentil mapping has become important as these markers are codominant and more robust than RAPD and AFLP.

Fratini et al. (2007) developed an intrasubspecific genetic map of *Lens*, using 195 molecular markers (71 RAPDs,39 ISSRs,83AFLPs, and 2 SSRs) and 3 morphological markers in an F_2 mapping population. Genetic map consisted of 10 linkage groups covering 2172.4 cM with an average marker distance between markers 15.87cM.A predominantly gene-based genetic linkage map of lentil was constructed by Phan et al. (2007) from an F_5 population using 79 intron-targeted amplified polymorphic (ITAP) and 18 genomic SSR markers, developed from *Medicago truncatula* EST database, at maximum recombination fraction of 0.32 (LOD = 9). Of the 91 mapped markers, 75 were genic, whereas 16 were genomic SSR markers. Six markers remained unlinked. The map comprised seven linkage groups which spanned a total of 928.4 cM and linkage groups varied in length from 80.2 to 274.6 cM. Twelve per cent of the markers were segregated in a distorted fashion.

The lentil map developed by Tullu et al. (2008) comprised of 207 markers (144 AFLP, 54 RAPD and 9 SSRs) distributed over 12 linkage groups covering 1868 cM with an average distance between adjacent markers of 8.9 cM. Chi square analysis was run on 271 markers out of which 62 marker loci significantly deviated from the expected 1:1 segregation ratio (P> 0.05).An intraspecific lentil map of F_7 derived RIL population (206 individuals) was constructed by Saha et al. (2010a), that included 161 polymorphic markers including 23 SSRs, 30 RAPDs and 108 SRAPs. The constructed linkage map comprised 14 linkage groups that spanned 1565.2 cM with an average distance of 11.6 cM between marker loci in each linkage group. By using 94 individuals of the lentil RIL populations, Tanyolac et al. (2010) constructed a genetic linkage map using AFLP, ISSR, RAPD and some morphological markers. The map consisted of 11 linkage groups covering 1396.3 cM with an average marker density of 8.4 cM with a LOD score of 3 and maximum recombination value of 0.30.

Gupta et al. (2011) enhanced the linkage map of Phan et al. (2007) using population of 94 F_5 RILs with a total of 196 markers, including 15 *M. truncatula* EST-SSR/SSRs which were mapped and clustered into 11 linkage groups (LG) covering 1156.4 cM.

Genetic linkage map of *Lens* using 114 F_2 plants derived from the intersubspecific cross between L 830 and ILWL 77 was developed (Gupta et al. 2012). Of the 235 (34 SSR, 9 ISSR and 192 RAPD) markers used in the study, 199 (28 SSRs, 9 ISSRs and 162 RAPDs) were mapped into 11 linkage groups (LGs), varying between 17.3 and 433.8 cM and covering 3843.4 cM, with an average marker spacing of 19.3 cM.

Pote et al. (unpublished), genotyped 94 RILs using 127 markers, of which 119 (25 SSR, 4 ISSR and 90 RAPD) were mapped on ten linkage groups (LGs), varying in length between 14.8 to 240.2 cM and covering 855.3 cM with an average marker spacing of 7.18 cM Number of markers per linkage group varied from 2-30. Eight markers were unlinked, whereas 25 markers showed deviation from Mendelian segregation. The study reported assigning of 11 new SSRs on the linkage map.

Sharpe et al. (2013) mapped 454 SNPs on LR-18 population using Lc1536 SNP Illumina Golden Gate OPA. There were six SSRs and 537 contigs that could be mapped in LR-18. The map had seven linkage groups that likely represented the seven chromosomes in lentil. The linkage groups ranged from 58 cM to 226 cM and together they cover 834.7 cM.

A genetic map of lentil encompassing 57 SSRs and 261 SNPs was developed by using 126 RIL population of a cross Cassb X ILL2024 (Kaur et al., 2014). A total of 318 markers were attributed to 10 LGs covering 1,178 cM with an average marker density of 1 locus per 3.7 cM (ESM 3), while 7 remained unlinked.

Verma et al. (2015) developed 501 new genomic microsatellites and mapped them on RIL mapping population derived from a cross between Precoz x L830 comprising 126 F_8-RILs. Of these 216 were mapped on seven linkage groups at LOD4.0 spanning 1183.7cM with an average marker density of 5.48cM.

3. Mapping of quantitative trait loci (QTLs):

With the development of DNA markers and the construction of high-density linkage maps for many plant species, the QTL (quantitative trait loci) mapping technique has become a powerful tool for identifying genomic regions affecting quantitative traits by providing information on the map location, relative effect, gene action and dominance properties of each identified locus (Lander and Botstein 1989; Tanksley 1993). Genetic mapping of major quantitative resistance effects also enables pyramiding of multiple different resistance genes into one superior agronomic background. The use of molecular markers linked tothe major different resistance quantitative trait loci (QTL) would improve efficiency and speed of conventional resistance breeding strategies. In order to identify molecular markers significantly associated with resistance to A. Lentis, several genomic maps have been developed (Havey andMuehlbauer1989; Eujayl et al. 1998; Rubeena et al. 2003; Hamwieh et al. 2005; Tullu et al. 2008; Tanyolacet al. 2010). Although several major genomic regions controlling seedling resistance to A. Lentil shall been reported (Ford et al. 1999; Chowdhury et al.2001; Rubeena et al. 2003), those governing resistance at the pod/maturity stage havenot been identified. Also, the majorities of genome maps were created with mostly anonymous and dominant random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and intersimple sequence repeat (ISSR) markers (Eujayl et al.1997, 1998; Rubeena et al. 2003; Dura´n et al. 2004;Hamwieh et al. 2005). Nevertheless, the first generation maps served as foundations upon which to build more detailed genetic maps with greater utility. A detailed linkage map is required to define and distinguish QTL. Once major QTL have been uncovered, tightly linked markers may be validated for use in marker-assisted selection (MAS) and potentially even as a starting point for the positional cloning of the underlying functional resistance gene(s) (Haley and Andersson 1997).

Compared to the conventional breeding approaches for improved productivity under water limited environments, genomics offers great opportunities for dissecting quantitative traits into their single genetic determinants (Dudley 1993; Tanksley 1993; Lee 1995; Beavis and Kein 1996; Quarrie 1996; Young 1996; Prioul et al. 1997; Tuberosa et al. 2002). Identification of QTLs is paving the way for MAS (Ribaut et al. 2002; Morgante and Salamini 2003) and genomics-assisted pyramiding of the

beneficial QTL alleles. The identification and localization of genes in the genome which control variation for quantitative traits can greatly facilitate their use in breeding programmes. Thoday (1961) demonstrated that simply inherited gene markers can be used as tags to locate quantitative trait loci (QTL). The technique for identification of QTL by gene markers became more efficient with the availability of molecular markers. Molecular markers have been used to identify QTLs in several crops such as maize (Stuber and Moll 1972; Stuber et al. 1982; Edwards et al. 1987; Liu et al. 2012; Almeida et al. 2012), wheat (Cuthbert et al. 2008; Rebetzke et al. 2008a; Bennett et al.2012), rice (Vikram et al. 2011; Wei et al. 2012; Steele et al. 2012; Dixit et al. 2012), tomato (Tanksley et al. 1982; Paterson et al. 1988), common bean (Blair et al. 2006; Kwak et al. 2008; Pérez-Vega et al. 2010), soybean (Liu et al. 2005; Du et al. 2009; Abdel-Haleem et al. 2011; Zhang et al. 2012) and chickpea (Anbessa et al. 2006; Lichtenzveig et al. 2006; Anbessa et al. 2009; Gowda et al. 2011).Quantitative traits have been mapped in lentil for the purpose of associating molecular markers with phenotypic traits. However, very few molecular markers are used in lentil breeding because many of the molecular markers are not reproducible in multiple populations or are difficult and too expensive to screen (Ford et al., 2009). Information on the genetic control of important quantitatively inherited traits in lentil is limited. According to Tahir et al. (1994), four quantitative trait loci (QTLs) for DTF were detected on LG1, LG2, LG4, and LG7, whereas four QTLs for PH were on LG1, LG2, LG3, and LG5 of the interspecific map of Muehlbauer et al. (1995). Later, Sarker et al. (1999) identified a recessive allele and a polygenic system to control DTF in different crosses of lentil, and the flowering locus was assigned to LG5 of the interspecific map reported by Muehlbauer et al. (1995). Earliness is an adaptive trait and is one of the major factors of agronomic variation. Development of early maturing lines with optimum DTF combined with high and stable yield is a major breeding goal in lentil breeding. The term *earlinessgenes* was first used by Ford et al. (1981), and it was proposed to be different from genes controlling photoperiod response in wheat (*Triticum aestivum* L.).In an effort to develop cultivars with optimum seed size, mapping QTLs contributing to seed size-related traits in different genotypes and identifying markers closely linked to these genes represent a promising selection tool.

Thus, the purpose of this study was to construct a genetic linkage map and identify regions of the lentil genome associated with early vigour traits using RILs derived from a cross between cultivarWA8649090 x Precoz.

3. MATERIALS AND METHODS

The present investigation was carried out in the Department of Agricultural Biotechnology, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh. The material used and the methodology adopted to achieve the objectives of the investigation is given here under:

3.1 Plant material

An F_{10}recombinant inbred line (RIL) population of 94 individuals derived from an intraspecific cross of *Lens culinaris* ssp. *culinary* (WA8649090 x Precoz) was used for the construction of genetic linkage map. The material was procured from Fred Muehlbauer; Grain Legume Genetics & Physiology Research Unit, USDA-ARS, Washington State University, Pullman, USA. The parents differed from each other with respect to various agro-morphological traits including seed size and seed weight. The RILs were grown in greenhouse during 2016-17for collecting leaf samples for DNA extraction.

3.2 Methodology

3.2.1 Extraction of plant genomic DNA

Genomic DNA was isolated from young leaf tissue (0.5-1g) of the parents and F_{10}individuals using CTAB method (Murray and Thompson 1980). The leaf tissues were rinsed in deionized water, dried on tissue paper discs and ground to fine powder in liquid nitrogen in autoclaved pre-cooled pestles and mortars. The ground tissue was transferred to a separate 2 ml eppendorf tubes containing 800 µl of extraction buffer (2% CTAB, 100 mMTris, 20 mM EDTA, 1.4 mMNaCl and 1% PVP, pH 8.0) maintained at 60^{o}C in water bath and mixed vigorously. The mixture was incubated at 60^{o}C for 1 h with occasional mixing. An equal volume of chloroform-isoamyl alcohol (24:1) was added to the tubes followed by gentle mixing. The mixture was centrifuged at 10,000 rpm for 10 minutes at 4^{o}C. The aqueous phase was transferred to fresh tube, followed by addition of 500 µl of pre-chilled isopropanol. The contents of the tubes were mixed gently and the mixture was incubated at -20^{o}C for 1 h. DNA was precipitated by centrifugation at 10,000 rpm for 10 minutes using centrifuge (SIGMA, Laborzentrifugen, Germany).

The supernatant was drained and the resulting pellet was washed twice with 1 ml of 70% chilled ethanol. The pellet was dried in a stream of sterile air in a laminar air flow cabinet for 3-4 h. Dried DNA pellet was dissolved in 1 ml TE buffer (10 mMTris-HCl, 0.1 mM EDTA, pH 8.0). The dissolved DNA was treated with 1 µl of RNase (10 mg/ml). The quantity and quality of DNAwas estimated through micro-value fluorimeter (Eppendrof).

3.2.2 Primers used and their source:

Type of primers used for polymorphism survey and their source is described as under:

Table 3.1: Primers used and their source

Sr.No	Types of Primers	Source
1	*Lens* SSRs (published)	Hamwieh et al. 2005, 2009; Saha et al. 2010b
2	*Lens* SSRs (unpublished)	NIPGR, New Delhi
3	*Trifolium pratens* SSRs	Sato et al.(2005)

3.2.2.1 SSR analysis

Lens SSRs have length of the primers varied between 18 and 23 bases published and unpublished as shown above (Table 3.1) were used for polymorphism survey in parents. Each primer was tested for polymorphism at different annealing temperatures using DNA of both parents. The polymorphic SSRs were used for genotyping of F_{10}mapping family. For amplification of genomic DNA, a reaction mixture of 12.5 µl volume was prepared using 7.15 µl of sterilized distilled water, 1.0 µl template DNA (25 ng/ µl),0.5 µl of forward and 0.5 µl of reverse primer(5 µM), 1.0 µl $MgCl_2$ (25 mM), 1.25 µl 10 x PCR buffer (10 mMTris-Hcl, 50 mMKcl, pH 8.3),1.0 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (5U/ µl). The amplifications were carried out in Gene Amp PCR System 9700® (Applied Biosystems, CA, USA) and verity flex (Applied Biosystems, CA, USA) using protocol as given in Table 3.2.

The amplification products were electrophoresed in 3% agarose gel (HIMEDIA) and stained with ethidium bromide (0.5 µg/ml). The gels were visualized and photographed using the Gel-Documentation Unit (Labnet International inc., USA).

Table3.2: PCR conditions used for amplification of *Lens* genomic DNA

SSRs and their source	Steps	Temperature and time	Cycles
	Initial denaturation	94^0C for 3 minutes	
30 SSRs	Denaturation	94^0C for 30 seconds	
(Hamwieh et al. 2005)	Annealing	49-58 ^{0}C for 35 seconds	35
	Extension	72^0C for 1 Minute	
	Final extension	72^0C for 5 Minutes	
	Storage	4 ^{0}C for ∞	
	Initial denaturation	94^0C for 3 minutes	
14 SSRs	Denaturation	94^0C for 30 seconds	
(Hamwieh et al. 2009)	Annealing	52-55 ^{0}C for 30 seconds	30
	Extension	72^0C for 1 Minute	
	Final extension	72^0C for 5 Minutes	
	Storage	4 ^{0}C for ∞	
	Initial denaturation	94^0C for 3 minutes	
	Denaturation	94^0C for 35 seconds	
19 SSRs	Annealing	55^0C for 35 seconds	
(Saha et al. 2010b)	Extension	72^0C for 1 Minute	35
	Final extension	72^0C for 5 Minutes	
	Storage	4 ^{0}C for ∞	
	Initial denaturation	95^0C for 2 minute	
350 LCSSRs	Denaturation	95^0C for 50 seconds	
Lens SSRs (LCSSR series)(NIPGR, unpublished)	Annealing	65^0C for 20 seconds	
	Extension	72^0C for 50 seconds	
Touchdown profile	Denaturation	95^0C for 20 seconds	25
	Annealing	55^0C for 50 seconds	
	Extension	72^0C for 50 seconds	
	Final extension	72^0C for 10 Minutes	
	Stortage	4 ^{0}C for ∞	
	Denaturation	95^0C for 1 minute	
140 Tp SSRs (cross genera markers of *Trifolium pratens*)	Annealing	$50^0/52^0/55^0$C for 1 minute	
Sato et al.(20005)	Extension	72^0C for 60 seconds	35
	Final extension	72^0C for 5Minutes	
	Stortage	4 ^{0}C for ∞	

Table3.3: PCR conditions for RAPD/ISSR

Primer Type	Steps	Temperature and time	Cycles
	Initial Denaturation	94^0C for 5 Minutes	
RAPD/ISSR	Denaturation	94^0C for 1 Minute	
	Annealing	37^0C for 1 Minute	39 cycles
	Extension	72^0C for 2Minutes	
	Final extension	72^0C for 5 Minutes	
	Storage	4 ^{0}C for ∞	

3.2.3. Phenotyping of recombinant inbred lines (RILs):

A population of 94 RILs was phenotyped for four important quantitatively inherited early vigour related characters viz. seedling length, root length, shoot length, dry weight, number of leaves and number of branches. The material was grown in polyhouse at Palampur during 2016-17 using two replications.

Phenotypic data for four traits were recorded after 10 days from sowing in petri plate using paper top on method. Two replications were made by sowing 20 seeds per Petri plate. After 10 days phenotypic data of seedling length, root length, shoot length were recorded using scale according to their length (cm) and dry weight (g) of shoot and root was taken by separating shoot and root from cotyledon using weighing balance. The data on number of leaves and number of branches were recorded after 45 days in the polyhouse condition.

3.2.4 Generation of genotypic data

The presence and absence of each band of a particular molecular weight in RAPD, ISSR and SSR profiles of all the individuals was scored manually. For deducing relationship among individuals, each band of a specific molecular weight in the DNA profile of an individual was treated as locus/ marker. A data matrix with ‘A’

indicating the presence or absence of band in parent WA8649090 and 'B' the presence or absence of a particular band in parent Precoz was generated.

3.2.5 Linkage analysis:

Genotyping data were used for linkage analysis using JoinMap V4.0 (Van Ooijen 2006) softwere. Marker order was assigned using the regression mapping algorithm with maximum recombination frequency of 0.4 at minimum logarithm of odds (LOD) of 3 and jump threshold of 5.Ripple command was used after adding each marker locus to confirm marker order. The Kosambi mapping function was used to calculate the map distance. To detect segregation distortion, Chi square ($\chi 2$) values were calculated using Joinmap V4.0. Highly distorted and unlinked markers were excluded from analysis. The phenotypic data were analysed with ANOVA using CPCS1. The traits with non-significant σ^2_Gwere not considered for QTL analysis. Phenotypic correlations were calculated using means of 2 replications in each experiment.

3.2.6. QTL analysis:

Genotypic and phenotypic data of different traits under study were used for QTL analysis by using QTL Cartographer V.2.5 software (Wang et al. 2012) (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). Composite interval mapping (CIM) was performed by selecting Model 6 with the default window size 10 cM, control marker number 5, and backward regression method. QTLs with a positive or negative additive effect for a specific trait imply that the increase in the phenotypic value of the trait is contributed by the alleles from Precoz or WA8649090, respectively.

4. RESULTS AND DISCUSSION

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is an annual cool season, self-pollinated diploid (2n=2x=14) legume species with a relatively large genome size of 4,063 Mbps (Arumuganathan and Earle 1991). It is the 3rd most important food legume after chickpea and pea but has poor genomic resources. India accounts for the largest global area under lentil with 1.50 million ha area and 1.05 million tons production with yield of 681.8 Kg/ha (FAOSTAT, 2016).A low genetic variability and insufficient genetic information are the reasons until recently genetic maps of this species consisted of relatively small number of markers that covered small portion of lentil genome. Information on genetic control of important quantitatively inherited characters like plant vigour, flowering time and other phenological traits in lentil is limited. The early plant traits such as shoot length, root length, seedling length, dry weight, etc are important traits to study, as these are indicator of tolerance against drought. Development of molecular genetic markers and their use in QTL analysis has become a powerful approach for studying inheritance of complex traits. If QTL mapping is properly integrated in marker-assisted breeding programme, it is bound to accelerate development of varieties with desired traits. Therefore, the present work of mapping QTLs for early plant vigour traits provides the part of solution as far as the productivity and coverage of lentil crop is concerned.

The present investigation entitled "Molecular mapping of QTLs for early plant vigour traits in lentil" was carried out to construct a *Lens* linkage map using SSR, ISSR, RAPD markers and to identify quantitative traits loci linked to early plant vigour traits.

The results obtained on different aspects of present study have been presented and discussed below:

4.1 Linkage map construction using PCR-based markers

4.1.1 Genotyping of mapping population

Different types of SSR markers viz. *Lens* genomic SSRs (LcSSRs), red clover SSRs (RcSSRs), ISSRs and RAPD were used for parental polymorphism survey. Some of the already published SSRs (Hamweih et al. 2005; Phanet al. 2007; Saha et

al. 2010b; Gupta et al. 2012) were used as anchor markers. A summary of various types of polymorphic markers is presented in Table 4.1.

Table 4.1: Details of markers used for construction of intra-specific linkage map of lentil.

S. No.	Details of SSR markers	Total Primers	Polymorphic Markers	Percent Polymorphism	Markers Mapped
1.	**LcSSRs (NIPGR)**	350	45	12.85	42
2.	**Hamwieh et al. 2005, 2009**	44	15	34.10	15
3.	**Phan et al. 2007**	20	01	05.00	01
4.	**Saha et al. 2010b**	19	08	42.10	08
5.	**RcSSRs (Sato et al. 2005)**	145	24	16.55	24
6.	**ISSR**	25	03	12.00	03
7.	**RAPD**	250	172	68.8	172
	Total	**853**	**268**	**31.41**	**265**

In the present study, *Lens* SSRs (both published and unpublished) along with red clover (*Trifolium pratense*) were used for screening of parental DNA to detect polymorphism. Eight hundred fifty-three polymorphic SSR primers yielded 268 clear and scorable markers. The microsatellite markers identified in this study were highly polymorphic. Of all the scored SSR markers, 265 (98.88 %) were mapped on seven linkage groups, whereas three of the markers were found unlinked.

In marker-assisted breeding, co-dominant markers are effective in identifying desirable homozygous genotypes at early stages of selection (Hamwieh et al. 2005). Simple sequence repeats are tandem repeats of one to six nucleotides present in all eukaryotic genomes (Katti et al. 2001). Given their codominant inheritance, high polymorphism, and abundant distribution throughout genomes, SSRs have been used widely for genetic mapping, comparative analysis, and QTL analysis in plants (Varshney et al. 2005). Extensive efforts have been made to develop SSR markers in lentil through construction of enriched genomic library (Verma et al. 2014, 2015;

Andeden et al. 2015). However, the total number of currently available SSR markers is insufficient for genetic analysis in lentil. Moreover, such experimental approaches to develop SSR markers are laborious, time-consuming and costly. Therefore, there is a dire need of additional SSR markers for lentil genome research and breeding. With the ever-increasing number of DNA sequences available in public databases, genomic sequences provide a more rapid and economic method for developing SSR markers. Based on SSRs developed from the genome sequence, high-density genetic linkage maps can be constructed (Thudi et al. 2011).

In lentil, very few (about 200) genomic SSR markers have been developed and used for map construction (Duran et al. 2004; Hamwieh et al. 2005, 2009; Saha et al. 2010b) which are not enough for applications in lentil genomics. More recently a set of 122 and 360 new genomic SSR markers were reported by Verma et al. (2014) and Andeden et al. (2015), respectively. Further, a big repertoire of 501 *Lens* SSR was developed by Verma et al. (2015) using two microsatellite genomic libraries enriched for (GA/CT) and (GAA/CTT) motifs.

Though SSR markers are an important tool, a significant number of genomic SSR markers is still limited in lentil and only few of them have been used for map construction. Although several intra- and interspecific maps of lentil are available with different mapping populations (Eujayl et al. 1998a; Rubeena et al. 2003; Duràn et al. 2004; Kahraman et al. 2004a&b; Hamwieh et al. 2005; Gupta et al. 2012), but they have been constructed using morphological markers, RFLP, RAPD, AFLP and only 39 genomic SSR markers. Recently, Verma et al. (2015) mapped 216 SSR markers on lentil linkage map and this was the highest number of SSR markers mapped till date in lentil. Further we also exploited the cross transferable nature of SSRs in our study by utilizing red clover SSR primers screened for polymorphism survey.

In the present study, 853 SSR, RAPD and ISSRs markers [433 (*Lens* genomic SSRs) + 145 (SSRs from other legumes viz. red clover) + 25 ISSR + 250 RAPD] were used for construction of *L. culinaris* linkage map. Of these, 268 (31.41 %) were found to be polymorphic between the parents of the RIL mapping population. Of these 268 polymorphic markers, 69 were genomic SSRs of Lens of other legume species, 3 were from ISSR and 172 were from RAPD. The level of polymorphism

observed in our study was in agreement with varied levels of polymorphism observed in other legumes such as 22.1 % in chickpea (Radhika et al. 2007), 26.8 % in adzuki bean (Chaitieng et al. 2006), 27.02 % in soybean (Hwang et al. 2009) and 37.0 % in lotus (Yang et al. 2012). It has been documented that different molecular tools for genomic analysis and improvement could not be extended in legumes to a certain level due to their narrow genetic base (Gupta et al. 2012). However, the polymorphism detected in this study was also compared to other plants which varied from as low as 6.5 % in tomato (Shirasawa et al. 2010a), 23.2 % in cucumber (Zhang et al. 2012a) to as high as 32.8 % in *Catharanthus* (Shokeen et al. 2011) and 50 % in *Vitis* (Riaz et al. 2004).

A number of factors affect the level of polymorphism exhibited by the parents of the mapping population such as type of marker, type of cross (self- or cross-pollinated, inter- or intraspecific cross), type of population (F_2/BC/RIL) etc. Previously interspecific populations have been used for map construction in genus *Lens* (Havey and Muehlbauer 1989a; Muehlbauer et al. 1989; Weeden et al. 1992; Tahir et al. 1993, 1994; Vaillancourt and Slinkard 1993; Tahir and Muehlbauer 1994; Eujayl et al. 1998a) exhibit higher polymorphism. Interspecific mapping populations do not represent true recombination distances of the cultivated species (Causse et al. 1994; Lefebvre et al. 1995) and may lead to segregation distortion (Tadmor et al. 1987). Crossing within the cultivated species are preferred for breeding as they identify polymorphic markers within the cultivated gene pool (Menéndez et al. 1997) and may also minimize the problem of linkage drag which is often observed in the crosses involving wild species (Saliba-Colombani et al. 2000). Due to less segregation distortion intraspecific maps are recommended for QTL analysis and mapping (Havey and Muehlbauer 1989a). To date, only one comprehensive SSR based intraspecific map has been reported (Verma et al. 2015) and the present map will be one of the first fairly saturated map which would be useful in the genetic enhancement of this crop. Moreover, previously generated *Lens* maps differ from the current map with respect to number and type of markers, cross, population type, marker density and genome coverage (Havey and Muehlbauer 1989b; Weeden et al. 1992; Eujayl et al. 1998a; Rubeena et al. 2003; Durán et al. 2004; Hamwieh et al. 2005; Tullu et al. 2008; Tanyolac et al. 2010; Gupta et al. 2011; Gupta et al. 2012; Verma et al. 2015).

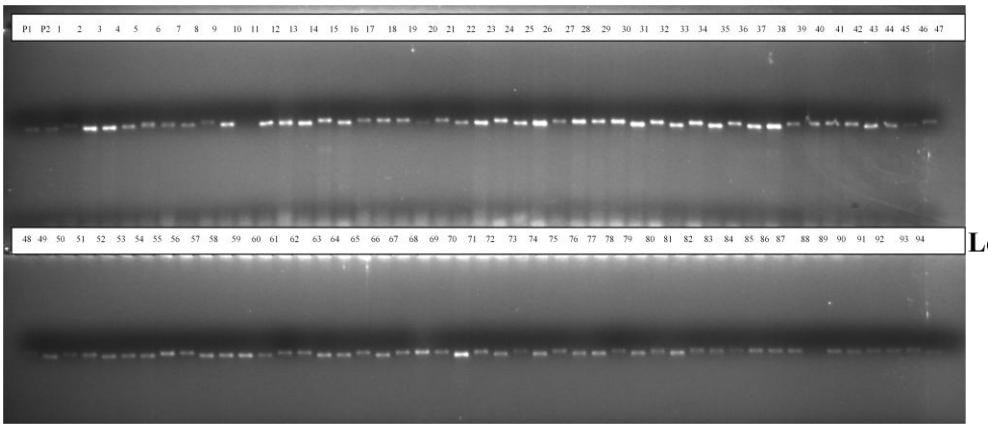


Fig 4.1 SSR banding profile using LcSSR 353 primer on P1 (WA8649090), P2 (Precoz) and 94 F_{10} RILs.

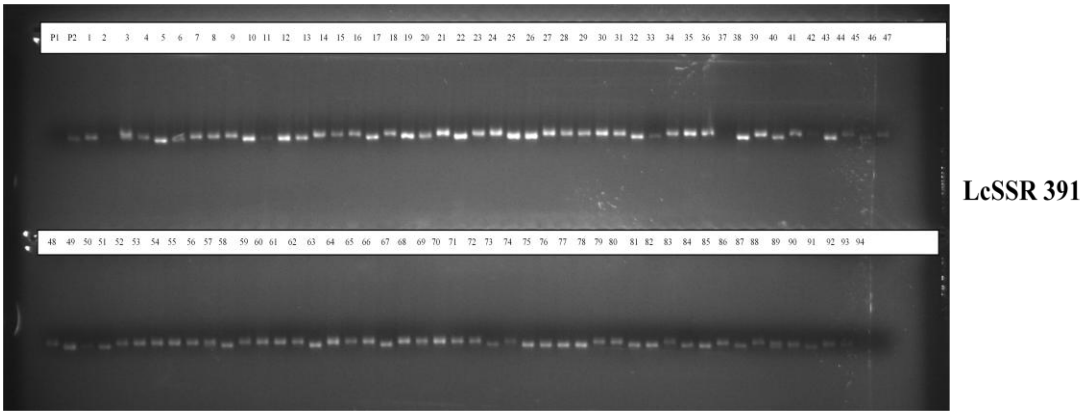


Fig 4.2 SSR banding profile using LcSSR 391 primer on P1 (WA8649090), P2 (Precoz) and 94 F_{10} RILs.

4.1.2 Construction of an intraspecific linkage map of lentil

A framework linkage map was constructed using the genotyping data of 268 polymorphic markers using JoinMap software, version 4.0 [van Oojen 2006; (as described in Chapter 3, Material and Methods, Section 3.3.3)]. A total of 265 markers were assigned positions on seven linkage groups (LGs) at LOD 4.0, based on the number of chromosomes of *L. culinaris* (2*n*=14, *n*=7). The generated linkage map of lentil using these markers spanned 809.4cM distance with an average marker density of 3.05 cM (Fig. 4.3). Of the 265 mapped markers included 42 LcSSR series, 15 markers of SSR series, 08 markers of GLLC series, 1 marker of Lup (Phan et al. 2007) series, 24 of RcSSRs, 172 of RAPD series and 3 of ISSR series. All the above markers were distributed across seven linkage groups and have been shown in Fig. 4.3. Based on the positions and groupings of the common SSR markers shared between the current genetic map and the previously published map of lentil (Hamwieh et al. 2005; Saha et al. 2010b; Verma et al. 2015), the LGs were numbered (LG1-LG7), accordingly.

The map length (809.4 cM) was comparable to other intraspecific linkage maps of lentil (784.1 cM; Rubeena et al. 2003), (1192 cM; Kahraman et al. 2004a), (1868 cM; Tullu et al. 2008), (1565.2 cM; Saha et al. 2010b), (1183.7 cM; Verma et al. 2015). In short, the average marker density of 3.05 cM observed in the present map is denser than the previous reported intraspecific lentil maps described above.

The map contained an average of 0.06 map positions per Mbp of genome (265 map positions/4063 Mbp), considering the lentil genome to be 4063 Mbp (Arumuganathan and Earle 1991). The details of number of markers mapped in a linkage group, region they spanned and the average marker density exhibited by them have been summarized in Table 4.2. The length of LGs varied from 25.6 cM in LG7 to 210.3 cM in LG3. The average marker density varied from 1.73 to 6.78 cM, with an average of 3.05 cM, indicating differing degrees of saturation of LGs. The average marker density on each linkage group revealed that the markers were randomly distributed (Fig. 4.3). Though the maximum number of markers was mapped in LG3, which harboured 76 markers, the average density was highest in LG1 (2.05 cM), which harboured 25 markers (Fig. 4.3).

Among the 7 linkage groups, each group differed from one another with respect to length and marker distribution. There were 4 large groups, 2 small groups and one smallest group LG7 which comprised of only 8 markers. As a result of random distribution of markers in the present study some groups were densely packed (LG3, LG6 and LG1), whereas LG7 and LG2 contained only 08 and 21 markers, respectively, which can be explained by the fact that SSRs are ubiquitously and randomly distributed in the plant genomes (Elsik and Williams 2001). Most of the markers were located on the centromeric region due to the lower recombination in these regions (Areshechenkova and Ganal 1999; Ramsay et al. 2000). The genomic origin of DNA sequences used for the SSR identification is also responsible for their unequal distribution on the groups and thus lead to less genome coverage (Tanksley et al. 1982).

Table 4.2 Distribution of 265 genomic markers on seven linkage groups of an intra-specific linkage map of lentil (*L. culinaris* WA8649090 x *L. culinaris* Precoz).

LGs	Markers Mapped	Map length (cM)	Average marker density (cM)	Skewed Markers (%)
LG1	56	115.3	2.05	15 (26.78)
LG2	25	169.5	6.78	08 (32)
LG3	76	210.3	2.76	9 (11.84)
LG4	22	38.1	1.73	02 (9.09)
LG5	21	108.7	5.17	17 (80.95)
LG6	57	141.9	2.48	17 (29.82)
LG7	8	25.6	3.2	03 (37.5)
Total	**265**	**809.4**	**3.05**	**71 (26.79)**

The markers were unevenly distributed on all the LGs, except for LG1 which showed cluster of 5 markers with 1 cM distance, Similarly LG3 and LG6 which showed cluster of 2 markers with 1 cM distance. The largest gap of 39.9cM was observed on LG2 followed by 31.1 on LG5 (Fig. 4.3). This may be due to the occurrence of fewer marker polymorphisms in these gaps or regions thus resulting in lower marker density. As homozygous regions possess lower recombination frequency and thus may be a possible explanation of low density of markers in these distal regions (Castiglioni et al. 1999; Souza et al. 2013).

The maximum and minimum distance between markers was 39.9 cM (LG2) and 0.0 cM (LG2,LG3 and LG6), respectively. The distribution of markers between linkage groups was unequal.

The differences in the crossing-over frequency can influence marker density in a linkage group. Tanksley et al. (1992) explained the uneven marker distribution with the reasoning that centromeres and centromeric heterochromatin and in some instances telomeres experience up to ten-fold less recombination. Heterogeneity in recombination along the genome has implications on the development of high resolution linkage maps as the latter are much easier to develop for regions of higher recombination. On the other hand, mapping of recombination suppressed regions requires much larger progeny sizes to allow the rare recombination events to occur, which is necessary for the construction of fine maps.

A non-random distribution of markers due to centrally located clusters has been reported in barley (Langridge et al. 1995), sugar beet (Halldén et al. 1996) and chickpea (Winter et al. 2000). Sometimes, the apparently random marker distribution is due to a low number of markers; when more markers were added to the map, clusters became evident, such as SSRs in soybean (Akkaya et al. 1995; Cregan et al. 1999). In cereals, in which cytogenetic markers are available, the crossing over frequency in the distal regions of the chromosomes has been shown to be higher than in the regions proximal to the centromere (Lukaszewski 1992; Alonso-Blanco et al. 1993).

Previous *Lens* maps (Havey and Muehlbauer 1989b; Weeden et al. 1992; Eujayl et al. 1998a; Rubeena et al. 2003; Durán et al. 2004; Hamwieh et al. 2005; Tullu et al. 2008; Tanyolac et al. 2010; Gupta et al. 2011; Gupta et al. 2012 and Verma et al. 2015) differed with respect to marker type and number, genome coverage and marker density. The present map covered 809.4 cM of the lentil genome with a mean marker distance of 3.05 cM, which was at variance with those reported in the previous maps (6.0 cM, Eujayl et al. 1998a; 6.9 cM, Rubeena et al. 2003; 15.87 cM, Durán et al. 2004; 2.7 cM, Hamwieh et al. 2005; 13.5 cM, Phan et al. 2007; 8.9 cM, Tullu et al. 2008; 8.4 cM, Tanyolac et al. 2010; 11.6 cM, Saha et al. 2010b; 7.1 cM, Gupta et al.2011 and 19.3 cM, Gupta et al. 2012; 5.48 cM, Verma et al. 2015).

Several factors including population size and the number of markers used in the analysis may contribute to differences in map coverage (Laucou et al. 1998). Differences in the genetic background of mapping populations could account for some marker rearrangements resulting in variation of size and number of linkage groups in the current and previous genetic maps. According to Paran et al. (1995), the level of stringency (LOD score and recombination frequency), number of markers, and size of mapping populations also have effects on the size and number of linkage groups.

As the physical size of the lentil genome was estimated to be 4,063 Mpb (Arumuganathan and Earle 1991), one cM of the present map translates on the average to 3.39 Mbp on an average compared to the value of 3.8 Mbp/ cM and 1.88 Mbp/ cM calculated in the maps obtained by Eujayl et al. (1998a) and Durán et al. (2004), respectively.

Considering the number of lentil maps with different backgrounds, integration of individual maps to develop a consensus map becomes imperative. This map can increase marker density, allowing comparison of locations of genes of interest across maps as was evidenced in common bean (Freyre et al. 1998) and barley (Marcel et al. 2007).

A high level of correspondence was observed for location of mapped SSRs in the present study and the earlier studies (Table 4.3). For example, SSR80 (LG1) was mapped on the same linkage group by Hamwieh et al. (2005) and Verma et al. (2015). SSR107 and SSR184 were assigned position on LG2 in the present linkage map which is in complete agreement with Hamwieh et al. (2005), Phan et al. (2007), Tullu

Table 4.3: Correspondence between linkage groups obtained in the present study and those obtained in previous studies on the basis of mapped anchor markers

S. No.	Marker locus	Present study	Hamwieh et al. (2005)	Phan et al. (2007)	Tullu et al. (2008)	Saha et al. (2010a)	Gupta et al. (2011)	Gupta et al. (2012)	Fedoruk et al. (2013)	Kaur et al. (2013)	Sharpe et al. (2013)	Verma et al. (2015)
1.	SSR19	6	VI	-	-	-	-	-	-	VI	-	VI
2.	SSR33	3	III	I	-	-	I	-	-	I	-	-
3.	SSR80	1	I	-	-	-	-	-	II	IV	-	I
4.	SSR107	2	II	II	-	-	II	II	-	II	-	II
5.	SSR113	1	VIII	-	IV	-	-	VIII	-	III	-	-
6.	SSR156	6	VII	V	-	-	V	V	V	V	V	-
7.	SSR184	2	II	II	II	-	II	II	-	II	-	II
8.	SSR204	4	I	IV	-	I	III	-	II	IV	I	-
9.	SSR302	1	I	IV	I	-	III	-	II	IV	-	I
10.	GLLC108	3	-	-	-	II	-	-	-	-	-	-
11.	GLLC511	5	-	-	-	VIII	-	-	-	-	-	-
12.	GLLC527	4	-	-	-	VIII	-	VIII	-	-	-	-
13.	GLLC562	3	-	-	-	II	-	-	-	-	-	-
14.	GLLC591	5	-	-	-	XII	-	-	-	-	-	-
15.	GLLC609	3	-	-	-	II	-	VI	-	-	-	-

et al. (2008), Gupta et al. (2011), Gupta et al. (2012), Kaur et al. (2013) and Verma et al. (2015). Similarly, GLLC markers (GLLC556 and GLLC607) were present on the same linkage groups as reported by Saha et al. (2010a). Further, they located GLLC511 on LGVIII, however in present study it was present on LG5. This might be due to the presence of large number of markers on current linkage map than previous maps.

4.1.3 Segregation distortion

The data matrix prepared with the help of genotypic codes for an RIL population, as mentioned earlier in section 3.3.2, was used for the construction of a genetic linkage map. During map construction, many markers showed deviation from the expected Mendelian segregation ratio. The $\chi 2$ test was performed for 268 markers to assess the goodness-of-fit for the expected Mendelian segregation ratio. The values obtained for each marker is tabulated in Table 4.4. Of the 268 polymorphic markers, 194 (73.20 %) followed the expected segregation ratio, whereas 71 loci (26.79 %) showed segregation distortion (Table 4.4). Among these 71 loci, 36 markers (50.70 %) showed slight deviation (* and **) from the expected ratio while 35 loci (49.29 %) exhibited significantly high segregation distortion (*** or above) (Table 4.4). The degree of skewness in these loci varied significantly. Of the 71 loci that showed segregation distortion, 32 (45.07 %) were skewed towards the parent Precoz, whereas approximately equal number of markers i.e. 39 (54.92 %) markers skewed towards WA8649090 parent. Further, all the distorted loci were mapped and most of these markers resided on LG3, LG5, LG1 and LG6 (Table 4.4).

Table 4.4: The genomic markers used in the present study for linkage map construction.

LG1

S. No.	Locus	Chi square value ($\chi 2$)	Segregation distortion	Distorted towards WA8649090/ Precoz
1	LcSSR602	4.06	**	WA8649090
2	LcSSR637	5.26	**	Precoz
3	LcSSR352	6.72	***	Precoz
4	SSR34	13.79	******	Precoz
5	LcSSR669	25.81	*******	Precoz
6	LcSSR471	21.77	*******	WA8649090
7	SSR80	0.73	-	-
8	TPSSR45	0.1	-	-
9	LCSSR463	8.38	****	Precoz
10	RCS1303c	3.45	*	Precoz
11	OPN5	0.91	-	-

12	OPR6a	4.35	**	Precoz
13	OPI16e	2.09	-	-
14	OPH19	0.87	-	-
15	SSR302	0	-	-
16	OPN6c	1.11	-	-
17	OPS14	0.54	-	-
18	OPE19	0.1	-	-
19	OPC12b	3.11	*	Precoz
20	OPJ1a	0.27	-	-
21	OPI11a	1.09	-	-
22	OPR15b	0.04	-	-
23	RCS4659a	0.71	-	-
24	OPI16b	14.09	******	WA8649090
25	OPI16a	5.26	**	WA8649090
26	LcSSR618	8.01	****	WA8649090
27	OPI16d	5.69	**	WA8649090
28	OPI16c	2.13	-	-
29	OPB15	2.28	-	-
30	OPC12a	1.57	-	-
31	RCS2918a	2.28	-	-
32	SSR204	0.95	-	-
33	OPL7	0.17	-	-
34	ubc890_8	0.71	-	-
35	ubc840_3	0.68	-	-
36	ISSR11b	1.06	-	-
37	LCSSR176	0.04	-	-
38	ubc822_3	0.1	-	-
39	ubc822_9	0.87	-	-
40	E1M3_4	0	-	-
41	ubc715_2	2.42	-	-
42	ubc808_3	1.46	-	-
43	ubc897_10	1.36	-	-
44	ubc890_5	0.38	-	-
45	ubc807_1	0.04	-	-
46	ubc771_1	0	-	-
47	ubc840_5	1.06	-	-
48	ubc807_3	0.56	-	-
49	ubc890_10	0.04	-	-
50	ubc842_7	1.53	-	-
51	ubc840_10	2.23	-	-
52	ubc809_3	3.11	*	Precoz
53	ubc840_17	0.87	-	-
54	E3M1_4	0.4	-	-
55	E2M1_13	0.22	-	-
56	LcSSR364	2.18	-	-

*** and **** : Slight deviation ; ***** or above**: High segregation distortion

LG2

S. No.	Locus	Chi square value ($\chi 2$)	Segregation distortion	Distorted towards WA8649090/ Precoz
1	LcSSR511	15.74	*******	WA8649090
2	OPI8b	0.53	-	-
3	E3M1_6	0.19	-	-
4	ubc808_5	0.19	-	-
5	LcSSR420	0.73	-	-
6	ubc571_1	2.42	-	-
7	LcSSR646	25.6	*******	WA8649090
8	ubc822_7	3.45	*	Precoz
9	P4M3_2	1.25	-	-
10	SSR202	10.33	****	Precoz
11	ubc841_5	4.38	**	Precoz
12	cs31_1	3.32	*	Precoz
13	cs31_3	7.35	***	Precoz
14	SSR107	0.11	-	-
15	RCS7205	0.71	-	-
16	SSR184	1.36	-	-
17	OPI14	0.53	-	-
18	RCS3753a	1.33	-	-
19	LCSSR321	1.33	-	-
20	LCSSR138	1.57	-	-
21	LcSSR369	1.33	-	-
22	OPAB5b	0.56	-	-
23	LcSSR630	1.53	-	-
24	LcSSR357	0.87	-	-
25	GLLC563	9.57	****	Precoz

and ** : Slight deviation ; ***** or above**: High segregation distortion

LG3

S. No.	Locus	Chi square value ($\chi 2$)	Segregation distortion	Distorted towards WA8649090/ Precoz
1	LcSSR371	0.56	-	-
2	LcSSR536	2.09	-	-
3	SSR96	38.3	*******	WA8649090
4	OPI11b	1.09	-	-
5	OPU14c	2.04	-	-
6	ubc807_5	0.1	-	-
7	LCSSR83	0.19	-	-
8	LcSSR423	18.89	*******	WA8649090
9	GLLC562	0.19	-	-
10	RCS3753c	0.27	-	-
11	OPC11	0.01	-	-
12	OPM7a	0.04	-	-
13	OPN11b	0	-	-

14	ubc763_1	1.22	-	-
15	ubc881_1	0.12	-	-
16	ubc809_5	0.1	-	-
17	ubc840_16	0.01	-	-
18	RCS0004	1.11	-	-
19	SSR48	0	-	-
20	RCS6502b	0.04	-	-
21	ubc840_8	0.1	-	-
22	LCSSR314	0.68	-	-
23	RCS5453a	0.68	-	-
24	OPI11e	0.39	-	-
25	OPU2b	0.18	-	-
26	P2M1_8	0.71	-	-
27	OPC12c	0	-	-
28	OPS20a	1.11	-	-
29	LcSSR641	4.38	**	Precoz
30	ubc715_1	0.53	-	-
31	LCSSR66	0.17	-	-
32	ubc440_1	0.01	-	-
33	ubc808_12	0.17	-	-
34	GL101	15.07	******	WA8649090
35	LcSSR373	0.87	-	-
36	ubc841_3	1.3	-	-
37	LcSSR353	0.55	-	-
38	ISSR11a	0.68	-	-
39	LcSSR621	1.82	-	-
40	OPM9d	0.04	-	-
41	OPM9c	0.04	-	-
42	ubc840_4	0.38	-	-
43	ubc808_7	0.1	-	-
44	LcSSR531	19.88	*******	WA8649090
45	LcSSR640	0.27	-	-
46	ubc548	1.53	-	-
47	OPW16a	0.17	-	-
48	OPS11b	2.72	*	WA8649090
49	LcSSR628	2.13	-	-
50	TPSSR46c	0.3	-	-
51	OPR14	0.3	-	-
52	OPW16b	0.17	-	-
53	OPR16b	0	-	-
54	LCSSR316	0.27	-	-
55	RCS5453b	0.54	-	-
56	SSR113	0.04	-	-
57	RCS6958	0.04	-	-
58	OPM13	0	-	-
59	RCS6676	0.31	-	-
60	SSR191	0	-	-
61	P8M1_9	4.25	**	WA8649090

62	GLLC527	0.54	-	-
63	RCS3753b	0.54	-	-
64	OPU2a	0.04	-	-
65	GLLC609	0.19	-	-
66	OPR6c	0.89	-	-
67	E3M3_3	1.14	-	-
68	ubc808_6	0.53	-	-
69	E3M3_4	0.18	-	-
70	P4M2_2	0.01	-	-
71	LcSSR418	13.17	******	WA8649090
72	SSR33	0.05	-	-
73	ubc502_2	0.1	-	-
74	LcSSR616	0.54	-	-
75	LcSSR354	2.72	*	WA8649090
76	LcSSR383	0.17	-	-

and * : Slight deviation ; ***** or above**: High segregation distortion

LG4

S. No.	Locus	Chi square value (χ2)	Segregation distortion	Distorted towards WA8649090/ Precoz
1	OPI8a	3.88	**	Precoz
2	RCS4659b	0.17	-	-
3	OPS1b	0.04	-	-
4	OPR16a	0.73	-	-
5	OPAB18a	0.27	-	-
6	OPAB18b	0.87	-	-
7	GLLC591	0.54	-	-
8	RCS6008	0.39	-	-
9	OPH15	1.6	-	-
10	OPZ17	2.39	-	-
11	OPJ17a	1.33	-	-
12	OPJ17b	0.27	-	-
13	P2M2_15	0.89	-	-
14	OPR6b	0	-	-
15	ubc808_9	1.3	-	-
16	ubc840_12	0.17	-	-
17	OPS1a	2.42	-	-
18	P2M2_12	0.01	-	-
19	P5M2_3	0.86	-	-
20	OPO13a	7.19	***	WA8649090
21	ubc841_4	0.29	-	-
22	RCS0899a	2.09	-	-

and * : Slight deviation ; ***** or above**: High segregation distortion

LG5

S. No.	Locus	Chi square value ($\chi 2$)	Segregation distortion	Distorted towards WA8649090/ Precoz
1	SSR28	6.87	***	WA8649090
2	LcSSR458	0.31	-	-
3	ubc840_6	1.6	-	-
4	OPAB5a	0.11	-	-
5	SSR156	0.04	-	-
6	ubc809_8	9.78	****	WA8649090
7	cs54_2	9.57	****	WA8649090
8	PM17	8.71	****	WA8649090
9	OPI11c	13.17	******	WA8649090
10	LCSSR290	19.17	*******	WA8649090
11	OPN11a	16.35	*******	WA8649090
12	OPR8	20.6	*******	WA8649090
13	TPSSR46b	17.02	*******	WA8649090
14	GLLC511	19.88	*******	WA8649090
15	OPS20b	14.4	******	WA8649090
16	ubc845_2	19.17	*******	WA8649090
17	P4M3_4	5.69	**	WA8649090
18	ubc449_2	15.04	******	WA8649090
19	ubc809_2	8.52	****	WA8649090
20	ubc807_6	3.97	**	WA8649090
21	LcSSR489	10.89	*****	Precoz

and *** : Slight deviation ; ***** or above**: High segregation distortion

LG6

S. No.	Locus	Chi square value ($\chi 2$)	Segregation distortion	Distorted towards WA8649090/ Precoz
1	LcSSR643	22.25	*******	WA8649090
2	LcSSR391	7.18	***	WA8649090
3	OPJ1b	1.82	-	-
4	OPM7b	1.82	-	-
5	ubc881_7	1.11	-	-
6	LcSSR366	0.38	-	-
7	E3M1_7	2.32	-	-
8	E3M1_8	0.56	-	-
9	OPM9a	0.27	-	-
10	ubc763_2	0	-	-
11	ubc809_1	0	-	-
12	cs48_1	1.39	-	-
13	cs52	9.24	****	Precoz
14	ubc808_10	5.69	**	Precoz
15	ISSR2b	11.71	*****	Precoz

16	GLLC108	9.67	****	WA8649090
17	OPS12b	0.87	-	-
18	OPS12a	0.1	-	-
19	RCS0899b	0.17	-	-
20	OPO13b	0.17	-	-
21	ubc809_7	0.71	-	-
22	ubc541_2	0.39	-	-
23	ubc842_1	0.04	-	-
24	OPI4a	0.4	-	-
25	RCS6502a	0.04	-	-
26	LcSSR611	9.45	****	Precoz
27	OPR16c	3.68	*	WA8649090
28	OPV17	0.29	-	-
29	SSR19	0.1	-	-
30	ubc841_2	1.82	-	-
31	OPO8	0.78	-	-
32	OPD5b	1.86	-	-
33	ubc781_2	1.06	-	-
34	ubc841_10	1.67	-	-
35	OPE1	0.04	-	-
36	OPN4	1.3	-	-
37	RCS1303a	0	-	-
38	ubc715_3	2.42	-	-
39	ubc440_2	0.87	-	-
40	OPS11a	5.15	**	Precoz
41	GLLC538	10.56	****	Precoz
42	OPC14	0.04	-	-
43	SSR90	0.01	-	-
44	RCS6615	0.04	-	-
45	OPM9b	0.87	-	-
46	OPP5a	0.87	-	-
47	P5M2_2	5.26	**	Precoz
48	OPD5a	0.01	-	-
49	OPN6b	0.4	-	-
50	ubc541_1	4.96	**	Precoz
51	OPI4b	4.44	**	Precoz
52	ubc890_2	2.78	*	Precoz
53	ubc840_11	0.7	-	-
54	OPP5b	15.04	******	Precoz
55	RCS1303b	14.09	******	Precoz
56	ubc834_6	3.68	*	Precoz
57	OPU14b	0.93	-	-

and *** : Slight deviation ; ***** or above**: High segregation distortion

LG7

S. No.	Locus	Chi square value ($\chi 2$)	Segregation distortion	Distorted towards WA8649090/ Precoz
1	P8M1_3	2.65	-	-
2	P8M1_10	2.28	-	-
3	P1M1_7	0.05	-	-
4	P4M1_6	0.01	-	-
5	P2M1_4	0.71	-	-
6	P1M1_5	3.05	*	WA8649090
7	P2M1_6	4.26	**	WA8649090
8	P2M1_5	8.91	****	WA8649090

and ** : Slight deviation ; ***** or above**: High segregation distortion

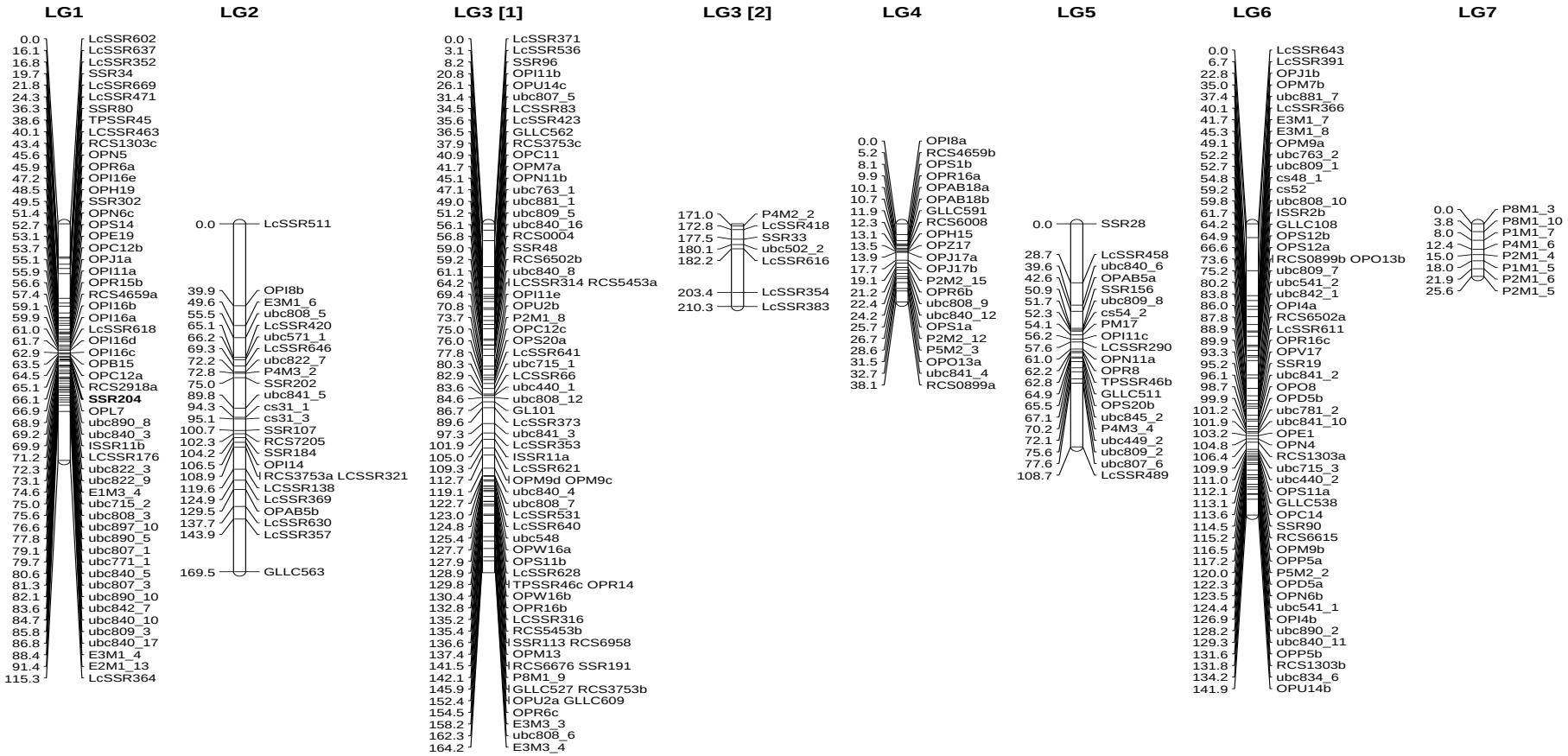


Fig 4.3. An intraspecific linkage map of *L. culinaris* based on RIL mapping population generated by crossing *L. culinaris* cv. WA8649090 × *L. culinaris* cv. Precoz. The map was generated with 265 polymorphic SSR, RAPD and ISSR markers using Join Map version 4.0 at a LOD value of 4.0, with Kosambi mapping function. A total of 265 markers were mapped on seven linkage groups (LG1 to LG7).

Segregation distortion is a common feature which is observed in most of the linkage maps in plants and has been attributed to difference in DNA content, structural rearrangement, self-incompatibility and deleterious recessive alleles (Quillet et al. 1995). In the present study, significantly high distortion 26.79 % (71/265) was exhibited by the markers. Different levels of segregation distortion in lentil mapping populations have been observed by different workers (9.5 % & 17.8 %, Hamwieh et al. 2005; 14.0 %, Rubeena et al. 2006; 12.0 %, Phan et al. 2007b; 20.0 %, Tullu et al. 2008; 48.0 %, Tanyolac et al. 2010; 22.0 %, Saha et al. 2010b; 23.81 %, Gupta et al. 2011; 33.85 %, Verma et al. 2015). However, as high as 83.3 % segregation distortion has been observed in previous lentil map using F_2 population. (Eujayl et al. 1997). Thus, it may be inferred that population type is not the only factor which is responsible for distortion but other factors such as chromosome loss during the process of crossing over, isolation mechanisms or the presence of other alien or viability genes may also affect the level of skewed segregation.

Segregation distortion in intraspecific crosses can be explained by small chromosomal rearrangements with little impact on fertility and chromosome pairing during meiosis, linkage to an incompatibility locus, or linkage to a lethal allele in gametes. A clustering of distorted loci has often been reported within the linkage groups constructed in several species.

4.2 Analysis of quantitative trait loci

The availability of genetic maps allows the localization and mapping of different agronomically important traits with the help of phenotypic data of segregating populations and in the identification of markers closely linked to the particular trait for marker-assisted selection and positional cloning. Linkage maps are useful for locating quantitative traits and understanding the genetic make-up of a crop. Genetic linkage maps in lentil have been constructed previously by many markers (Zamir and Ladizinsky 1984; Tadmor et al. 1987; Havey and Muehlbauer 1989; Tahir et al. 1993; Eujayl et al. 1997; Duran et al. 2004; Gupta et al. 2012b; Verma et al., 2015). Thus, the purpose of this study was to ascertain the genomic position, number and magnitude of QTLs affecting genetic variation for a early vigour traits in RIL populations derived from a cross between two lentil genotypes showing contrasting expression for these traits. The study thus provides valuable information on the feasibility of using QTLs in a marker-assisted selection scheme to identifying

genomic regions controlling plant early vigour traits. Identification of QTLs for important agronomic traits has been made possible in a number of plant species with the availability of polymorphic markers and linkage maps. Plant early vigour traits, like seedling length, root length, shoot length and dry weight are economically important quantitative traits, which are believed to be controlled by multiple genes. Germination rate and early seedling growth are the major seedling-vigour related traits. Rapid shoot and root growth were observed to be closely associated with seedling-vigour (Williams and Peterson 1973; Sahara et al. 1986). The early vigour traits are important for determining and establishment in the field as early as possible. It is generally considered that carbohydrates for early seedling growth before seedlings gain the ability of photo autotrophy are provided by breakdown of the starch stored in the endosperm. Thus, functionally, it should be expected that amylase activities are correlated with germination rate and early seedling growth in rice, as was reported previously (Williams and Peterson 1973; Sahara et al. 1986). The application of molecular-marker technology and QTL mapping has facilitated understanding of the genetic basis of many agriculturally important quantitative traits and phenomena. These studies showed that genetic variability of rapid early growth vigour is present among the lentil germplasm. To utilize this genetic variability in lentil breeding programme, it is essential to investigate the genetic control of early growth vigour. Knowledge of genetics of a trait helps to choose the parents and construct an appropriate breeding scheme (Sarker et al. 1999). Seed vigor is defined as “seed properties that determines potential for fast and uniform emergence and development of seedlings under a wide range of field conditions” (AOSA, 2002). Generally, low germination speed, high sensibility to stresses of seeds and seedlings during germination process and plants with slow, low and irregular growth or with less root development are typical characteristics of seed with low physiological potential. According to Hampton (2002) seed vigor has a high influence on establishment of initial population of plants as well as on their adequate development, what will affect crop yield. This way, seeds have low vigour could diminish percentage of emerged seedlings and seedling emergence speed, initial growth, leaf area, and dry mass accumulation. High seedling vigour helps the genotypes to suppress the weeds, which is a serious problem in large rain fed and upland areas in the tropics where dry seeding is practiced. The purpose of this study was to identify

quantitative trait loci (QTLs) underlying seedling vigour-related traits using a RIL mapping population derived from a cross between a *WA8649090* and Precoz.

Therefore, the present study QTL were using genomic as well as genic SSR markers and to identify the genomic regions associated with traits such as shoot length, root length, seedling length, dry weight, number of leaves and number of branches. The identification of these QTLs controlling such important traits would enable to analyse association between the mapped loci and traits and provide the basis for lentil genomics and breeding.

Table 4.5 Frequency distribution of different early vigour traits in 94 RILs derived from cross WA8649090 X Precoz.

A. Frequency distribution of Shoot length

SL	Midpoint	N	Cum. N	%	Cum. %
1	1.5	6	6	6.382979	6.382979
2	2.5	16	22	17.02128	23.40426
3	3.5	24	46	25.53191	48.93617
4	4.5	24	70	25.53191	74.46809
5	5.5	11	81	11.70213	86.17021
6	6.5	6	87	6.382979	92.55319
7	7.5	6	93	6.382979	98.93617
8	8.5	0	93	0	98.93617
9	9.5	1	94	1.06383	100

B. Frequency distribution of Root length

RL	Midpoint	N	Cum. N	%	Cum. %
0	0.5	2	2	2.12766	2.12766
1	1.5	19	21	20.21277	22.34043
2	2.5	29	50	30.85106	53.19149
3	3.5	29	79	30.85106	84.04255
4	4.5	6	85	6.382979	90.42553
5	5.5	7	92	7.446809	97.87234
6	6.5	1	93	1.06383	98.93617
7	7.5	0	93	0	98.93617
8	8.5	1	94	1.06383	100

C. Frequency distribution of Seedling length

SeL	Midpoint	N	Cum. N	%	Cum. %
0	1	1	1	1.06383	1.06383
2	3	8	9	8.510638	9.574468
4	5	32	41	34.04255	43.61702
6	7	27	68	28.7234	72.34043
8	9	15	83	15.95745	88.29787
10	11	8	91	8.510638	96.80851
12	13	2	93	2.12766	98.93617
14	15	0	93	0	98.93617
16	17	1	94	1.06383	100

D. Frequency distribution of Dry Weight

DW	Midpoint	N	Cum. N	%	Cum. %
0	0.015	45	45	47.87234	47.87234
0.03	0.045	34	79	36.17021	84.04255
0.06	0.075	9	88	9.574468	93.61702
0.09	0.105	3	91	3.191489	96.80851
0.12	0.135	2	93	2.12766	98.93617
0.15	0.165	0	93	0	98.93617
0.18	0.195	0	93	0	98.93617
0.21	0.225	1	94	1.06383	100

E. Frequency distribution of Number of leaves

NOL	Midpoint	N	Cum. N	%	Cum. %
90	93.75	1	1	1.06383	1.06383
97.5	101.25	0	1	0	1.06383
105	108.75	6	7	6.382979	7.446809
112.5	116.25	13	20	13.82979	21.2766
120	123.75	25	45	26.59574	47.87234
127.5	131.25	23	68	24.46809	72.34043
135	138.75	15	83	15.95745	88.29787
142.5	146.25	8	91	8.510638	96.80851
150	153.75	3	94	3.191489	100

F. Frequency distribution of Number of branches

NOB	Midpoint	N	Cum. N	%	Cum. %
2	3	7	7	7.446809	7.446809
4	5	16	23	17.02128	24.46809
6	7	39	62	41.48936	65.95745
8	9	18	80	19.14894	85.10638
10	11	9	89	9.574468	94.68085
12	13	3	92	3.191489	97.87234
14	15	1	93	1.06383	98.93617
16	17	1	94	1.06383	100

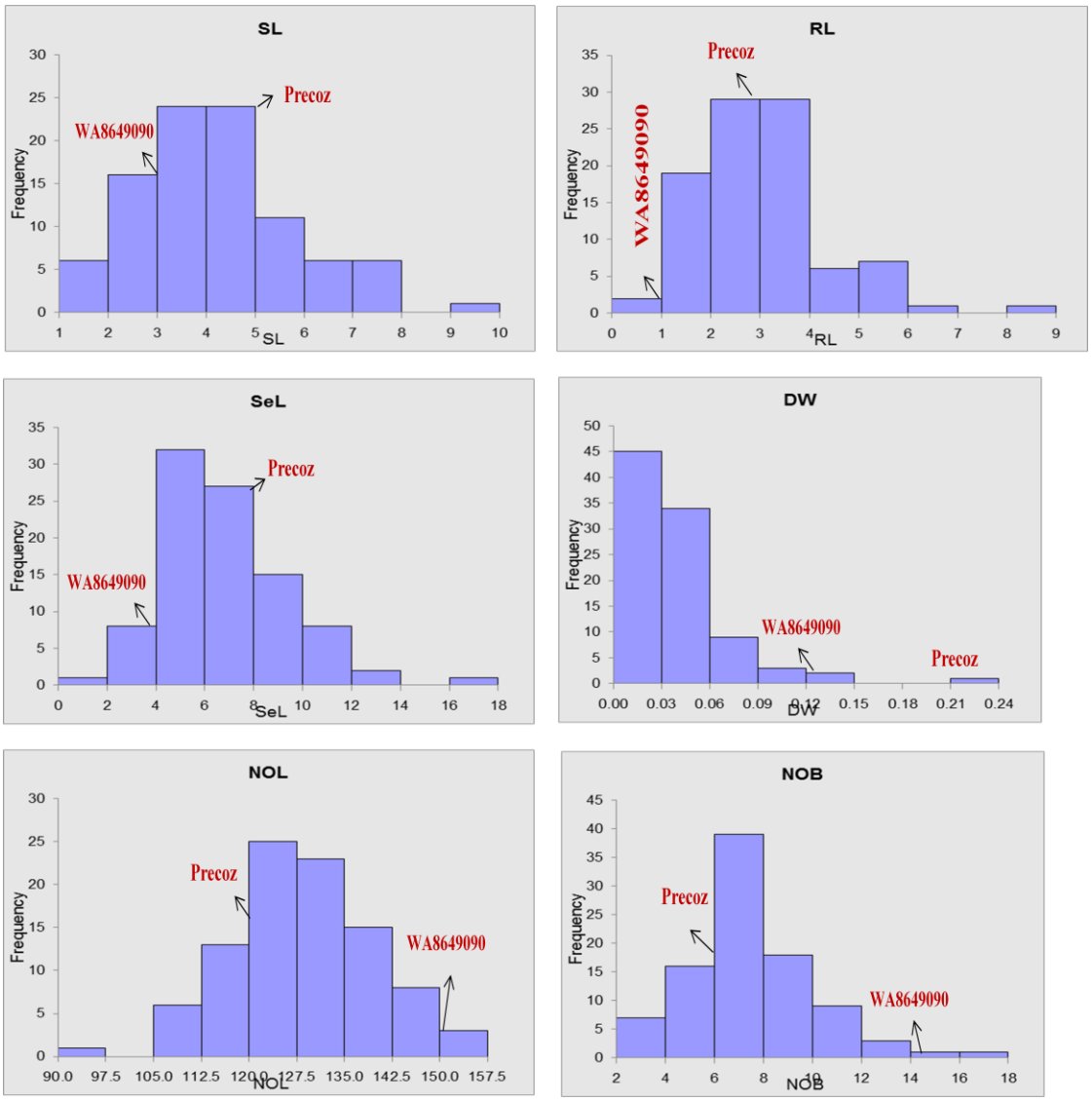
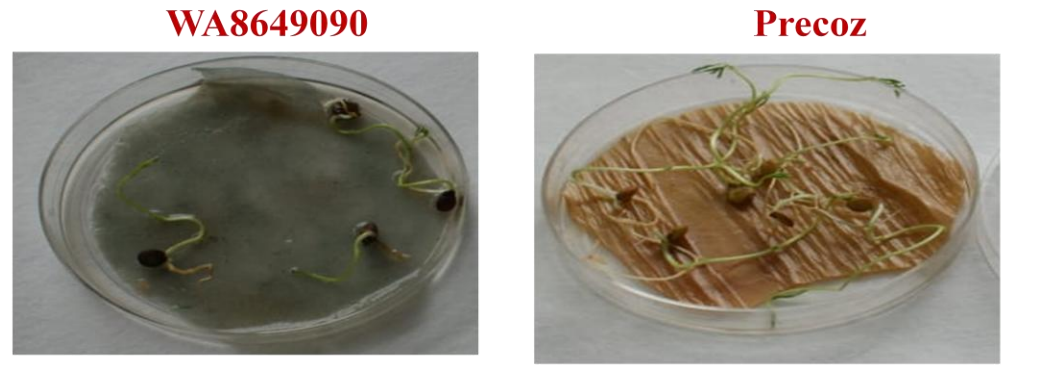
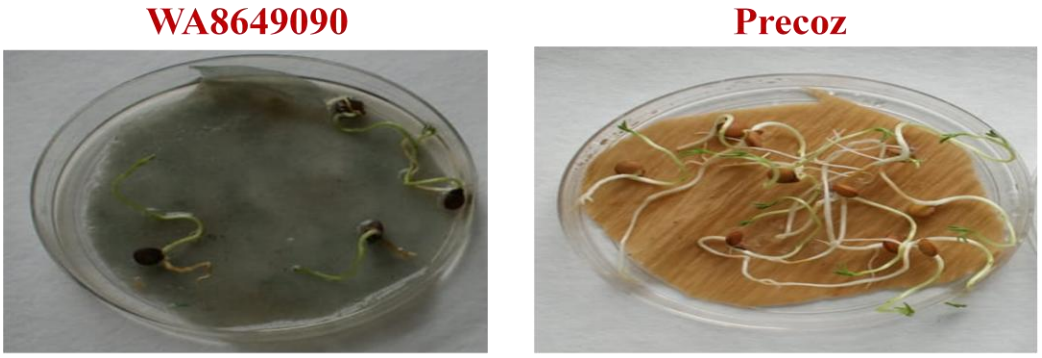


Figure 4.4 **Frequency distribution of different early vigour traits in 94 RILs derived from cross WA8649090 X Precoz.**





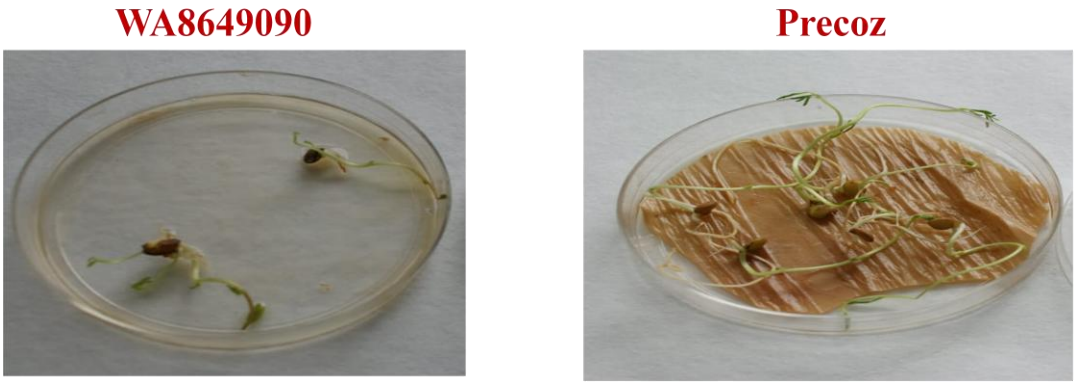


Figure 4.5. Early plant vigour traits of the two parental linesWA8649090 and Precoz

4.2.1 Analysis of morphological traits

(a) Analysis of variance and trait correlations

Significant differences for all the 6 measured traits were observed between the WA8649090 and the Precoz (Table 4.5). The ANOVA of 94 RILs revealed significant differences for almost all the traits. In general, the mean values of RILs were intermediate to that of parents. The values of RILs outside the parental range were also observed for the traits under study. This indicated that the alleles that increased phenotypic values were dispersed in both parental lines, even when their values differed markedly, thus indicating transgressive segregation for all traits (Table 4.7). These traits appeared to be quantitatively inherited as shown by the presence of nearly continuous distribution of mean phenotypic values of the traits for 94 RILs (Annexure-I).

The PCA of 94 RIL populations extracted two major principal components (eigenvalues > 1) that accounted collectively for 47.00 and 23.52 per cent of the variance for early plant vigour trait, respectively (Table 4.6). Principal component 1 explained 47 per cent of the data set variation, and was loaded positively with all measured traits. PC2 explained 23.52 per cent of the data set variation, and was positively loaded NOL and NOB traits only. Further, PCA showed that maximum contribution towards seedling length in the RILs followed by shoot length, root length, number of branches, dry weight and number of leaves.

Table 4.6. Principal component for different traits in RILs (WA8649090 × Precoz) Population

Value	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	2.820329	1.411603	0.767459	0.589262	0.323187	0.088159
% of Var.	47.00548	23.52672	12.79099	9.821041	5.386454	1.469317
Cum. %	47.00548	70.5322	83.32319	93.14423	98.53068	100

Table 4.7. Mean performance of parents, RILs, different traits in RIL (WA8649090 × Precoz) population

	SL	RL	SeL	DW	NOL	NOB
Mean	4.112553	2.966596	6.693511	0.035106	128.3968	7.434681
Std Dev.	1.644982	1.399033	2.596383	0.032087	10.9187	2.539174
Coeff. Var.	39.99904	47.15955	38.78955	91.39857	8.50387	34.1531
Minimum	1.5	0.3	1.55	0	97	2.5
Maximum	9.08	8.9	17.6	0.21	155	17.5
Range	7.58	8.6	16.05	0.21	58	15

4.2.2 QTLs for Early plant vigour

To understand the genetic and molecular basis of drought tolerance, developed genetic maps and extensive phenotyping data generated on RIL population were analyzed in detail for identification of both QTLs as well as the QTLs showing additive effects.

A total of 14 QTLs (LOD ≥ 3) were detected across the environments. (Table 4.8) In total, 4 QTLs were detected for seedling trait, 3 for dry weight and number of leaves, 2 for number of branches traits, 1 each for shoot length and root length traits. LG1 contained total of 6 QTLs (three for seedling length and one each for shoot length, root length and number of branches); LG2 contained only one QTLs (one for dry weight) while LG3 contained of 2 QTLs (1 each for number of leaves and number of branches); LG4 had a total of 2 QTLs (both for number of leaves trait), LG5 not contained any of the QTLs; LG6 contained 2 QTLs (Each for dry weight trait) and LG7 contained only one QTLs (one for seedling length trait) (Fig. 4.6). This uneven distribution of QTLs across LGs is in agreement with Fratini et al. (2007), Tullu et al. (2008) and Saha et al. (2013).

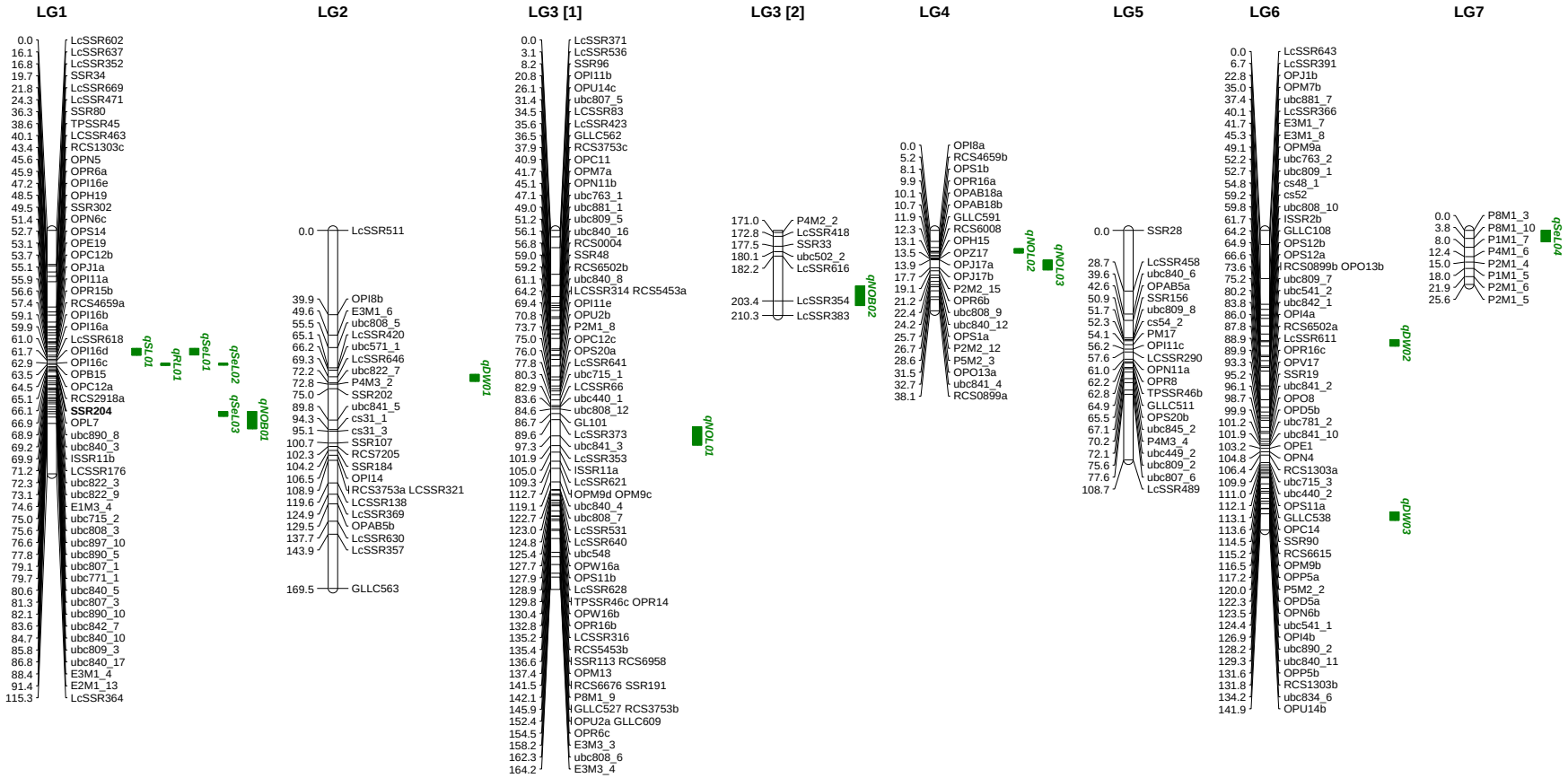


Fig 4.6. An intraspecific QTLlinkage map of *L. culinaris* based on RIL mapping population generated by crossing *L. culinaris* cv. WA8649090 × *L. culinaris* cv. Precoz. The map was generated with 265 polymorphic SSR, RAPD and ISSR markers using QTL cartographer Version 2.5 at a LOD value of 4.0, with Kosambi mapping function.

Table 4.8 QTLs for various early plant vigour traits identified using QTL Cartographer

Trait	LG	QTL Name	Position	LOD Score	Marker Interval	Additive	PVE
SL	1	qSL01	56.61	3.9	OPI11a-OPI16b	0.66	14.2
RL	1	qRL01	63.51	3.1	OPI16c-OPC12a	0.57	13.2
SeL	1	qSeL01	56.61	5.6	OPI11a-OPI16b	1.29	21.2
	1	qSeL02	63.51	4.8	OPI16c-OPC12a	1.34	19.4
	1	qSeL03	86.81	4.2	ubc809_3-E3M1_4	-1.17	14.2
	7	qSeL04	2.01	3.1	P8M1_3-P1M1_7	0.94	12.2
DW	6	qDW02	52.21	2.7	OPM9a-cs48_1	0.01	9.5
	6	qDW03	134.21	3.1	RCS1303b-OPU14	0.01	10.9
	2	qDW01	69.31	2.7	ubc51_1-ubc822_7	0.01	9.2
NOL	3	qNOL01	95.61	3.9	LcSSR373-LcSSR353	-5.14	21.4
	4	qNOL02	10.11	3.2	OPS1b-OPAB18b	3.80	11.4
	4	qNOL03	15.91	3.4	OPJ17a-P2M2_15	4.52	15.4
NOB	3	qNOB02	203.41	3.1	LcSSR616-LcSSR383	0.84	10.3
	1	qNOB01	86.81	3.7	ubc809_3-LcSSR364	-0.98	12.3

Trait dissection

Comprehensive QTL analysis provided an opportunity to analyse early plant vigour traits in depth. As QTL analysis was undertaken based on phenotypic data for 18 traits, collected during 1 year (season) at 1 location. Phenotypic variation explained (PVE) by QTLs ranged from 9.2 to 21.4 per cent (Table 4.8). The highest phenotypic variation (21.4 %) was explained by the QTLs for number of leaves. Although QTLs were detected on all linkage groups, six regions on LG1, LG2, LG3, LG4, LG6 and LG7 contained QTLs for several traits.

Early Plant Vigour Trait

The six major QTLs were detected on LG1 with a LOD score range of 3.1-4.8, explaining 12.3-21.2 per cent of the phenotypic variation (Table 4.8).Six traits analysed for shoot length characters, 1 significant QTL with up to 14.2 % PVE was present on LG1at LOD value of 3.9. This QTL had an additive effect of 0.66 g and

contributed by the allele from WA8649090 parent. Additive effect of these QTLs increases the shoot length of plant after introgression of the gene through Marker assisted breeding. Higher shoot length was conferred by Precoz allele at one loci*qSL01*. Similarly, other QTLs with up to 13.2 % PVE was present on LG1 at LOD value of 3.1. This QTL had an additive effect of 0.57 g and contributed by the allele from Precoz parent which governs the early plant vigour character for root. Additive effect of these QTLs increases the root length of plant after introgression of the gene through Marker assisted breeding. Precoz root increasing character located on it at loci *qRL01*.Out of 6, three highest seedling length was conferred by the alleles contributed by Precoz at three loci *viz.qSeL01*, *qSeL02* and *qSeL03*, also another number of branches was conferred by the allele contributed by WA8649090 at single loci *qNOB01*. In these QTLs three were detected on LG1 and one on LG7 with a LOD score range of 3.7-5.6, explaining 12.2-21.2 per cent of the phenotypic variation (Table 4.8). This means that seedling length and number of branches are influenced by QTLs additive effect. These positive additive effects (*qSeL01* and *qSeL02* has*1.29 and 1.34* respectively) governs an increasing seedling length and the negative additive effect(*qSeL03* and *qNOB01* has *-1.17* and *-0.98*) governs a decreasing seedling length and number of branches. In addition, three significant QTLs (two on LG6 and one onLG2) were detected for dry weight with a LOD score range of 2.7-3.1. These three QTLs had a same additive effect of 0.01 g and contributed by the allele from Precoz parent at three loci *viz.qDW02*, *qDW03* and *qDW01*, which governs the early plant vigour character for dry weight, explaining 9.2-10.9 per cent of the phenotypic variation.

Of the six traits analysed, major QTLs were identified for two traits, three QTLs for number of leaves and one for number of branches (Table 4.8). Number of leaves QTLs were present on LG3 and LG4 explaining 11.4-21.4 per cent of the phenotypic variation with at LOD score range of 3.2-3.9, while number of branches QTL was present on LG3 with 10.3 per cent of phenotypic variation explained at the 3.1 LOD value. These traits QTLs were contributed by the alleles from theWA8649090 which resulted in increased number of leaves and number of branches by their positive additive effect *viz.* 3.80 and 4.52 for *qNOL02, qNOL03*; 0.84 for *qNOB02* respectively. Also, negative additive effect leads to decreased number of

leaves by their -5.14 additive effect for *qNOL01*.Out of six traits; one significant QTL with up to 12.2 % PVE was present on LG7at LOD value of 3.1. This QTL had an additive effect of 0.94 g and contributed by the allele from WA8649090 parent. Additive effect of this QTL increases the seedling length of plant after introgression of the gene through Marker assisted breeding.

Candidate genomic regions for molecular breeding

In any breeding program, the traits to be considered as potential selection targets for improving yield under water limited conditions must be genetically correlated with yield, and should have a greater heritability than yield itself. Early seedling vigour (ESV) determines rapid, uniform emergence and the development of seedlings under a wide range of field conditions and it has been considered as one of the important characteristics that determines successful crop establishment (Zhang et al. 2005) under DSR(Direct Seeded Rice). Therefore, the objectives for breeding DSR genotypes should aim for high yield potential, environmental adaptation and ESV with favourable growth traits for weed suppression. Seedling vigour is an agronomical trait and sign of potential seed germination, seedling growth and tolerance to adverse climatic factors. On the other hand, it significantly improves the speed, uniformity and the final percentage of germination, and leads to ideal field appearance with good potential grain yield under direct-seeded conditions. Yet, few studies have been conducted under in vitro and in vivo conditions and also employing molecular approaches to utilize different types of molecular markers to map and tag traits of seedling vigour quantitative trait loci (QTLs) in different types of population. Genetic improvement of early seedling growth, information on genetic variation in traits related to ESV and also knowledge concerning the relationships among various seedling vigour traits as morphological and physiological traits is necessary.

Breeding for increased seedling vigour has been previously attempted by several workers, but progress has been slow due to the lack of suitable donors, limited knowledge on the genetics of seedling vigour, the involvement of several complex mechanisms, and complex genetic backgrounds and methods used for measurement.

Genomic regions containing QTLs for several traits are much valued by breeders. In this context, the detected QTLs were analysed and considered QTL

cluster/co-localized QTLs if they represent for more than three traits. Overall, one QTL cluster was identified. Among these, QTL Cluster 1 was located on LG1.

QTL Cluster 1 contained QTLs for shoot length trait (*q*SL 14.2 % PVE); Seedling length trait (qSeL01 21.2 % PVE; qSeL02 19.4 % PVE); Root length traits (qRL01 13.2 % PVE). Overall, the regionharbored 4 QTLs for three different traits explaining 13.2-21.2 % phenotypic variation. Therefore, introgression of this region will not only improve the shoot length trait but also root length and seedling length traits in lentil. Out of total 14 QTLs found in the study, 6 QTLs were clustered together. Thus, this cluster has been referred to as "*QTL-hotspot*" as this region contained several consistent QTLs with very high phenotypic variation explained and its introgression will definitely improve early plant vigour traits. On LG1 4 QTLs were identified about ≤ 8 cM that governs the different early plant vigour traits.

Quantitative trait loci (QTLs) affecting earliness, plant height, winter hardiness and resistance to ascochyta blight were identified in lentil (Gupta et al. 2011). The association of genomic regions with QTLs influencing early flowering and plant height using isozyme analysis was observed (Tahir et al. 1994). They detected QTLs on LG1 and LG2 which were involved in expression of both the traits.

Since no consensus genetic map of lentil exists, it is not always easy to compare the results obtained in earlier studies in relation to the chromosomal location of the QTL analyzed. The comparison based on localization of loci showed that current map exhibited some degree of alignment with previous maps. Differences in the genetic background of mapping populations could account for some marker rearrangements resulting in variation of size and number of linkage groups in the current and previous genetic maps. However, the number of anchor markers used was not sufficient to reach to a final conclusion. In lentil, some QTLs were tentatively identified among different studies. Using the single marker analysis and isozymes studies, a QTL related to days to flowering was previously located in linkage group 1, while QTL related correspondingly to days to flowering and to plant height in linkage group 5 (Tahir et al. 1993; Tahir and Muehlbauer 1995; Fratini et al. 2007). Fratini et al. (2007) localized genomic regions linked to flowering time in lentil on LGI and

LGX. Nine QTLs for plant height were identified on 5 different linkage groups in one environment, whereas two QTLs were detected for this trait in another environment (Tullu et al. 2008).

In this study, all the QTLs detected were found to be clustered across almost all the linkage groups, except LG5. Clustering of QTLs for various agronomic traits has been reported in many agriculturally important crops like sorghum (Lin et al. 1995), common bean (Blair et al. 2006), wheat (Quarrie et al. 2006), cotton (Qin et al. 2008), soybean (Xu et al. 2011), rice (Wang et al. 2012) *etc*. QTL clusters having more than one traits may have multiple effects on each other as they belong to the same genomic regions, like in this study QTLs for several traits were identified on a very small region on linkage groups. These QTLs clustered for several traits revealed that these regions were directly associated with grain yield. The clustering of QTLs can arise due to pleiotropic effect of a single regulatory gene (Aastveit and Aastveit 1993). The occurrence of pleiotropy could be explained in a way that certain traits are phenotypically correlated with each other due to the presence of certain genes coexisting in these QTLs. Fine mapping of these identified QTLs is the next step to understand whether linkage or pleiotropic effects are responsible for their clustering. As molecular markers are still limited in lentil, construction of second generation high density linkage map with the inclusion of SNP markers would increase the resolution of QTLs and provide a better picture of the occurrence of these QTLs for future genetic and genomic studies.

Overall, SSR markers and the linkage maps are extremely useful for plant breeding and crop improvement programs, wherein they have profound applications. Evidently, the map generated in the present study although covered a significant portion of the genome, a further saturation of this map with additional markers (SSRs or SNPs) is imperative for its efficient utilization. With the long-term goal of understanding the genetic basis of early plant vigour trait, the present study was focused on identification of major QTLs for 6 traits in lentil. In conclusion, it is envisaged that the present linkage map, fortified with 265 SSR markers and 14 QTLs for early plant vigour traits would provide a means to breeders for further genetic enhancement of the crop species. However, as discussed denser genetic linkage maps with large number of markers by the inclusion of SNPs would facilitate the

identification of more resolved and fine QTL positions which can significantly improve the resolution of identified QTLs for mapping. The knowledge of marker-trait association may also lead to the identification of genes influencing agronomic traits.

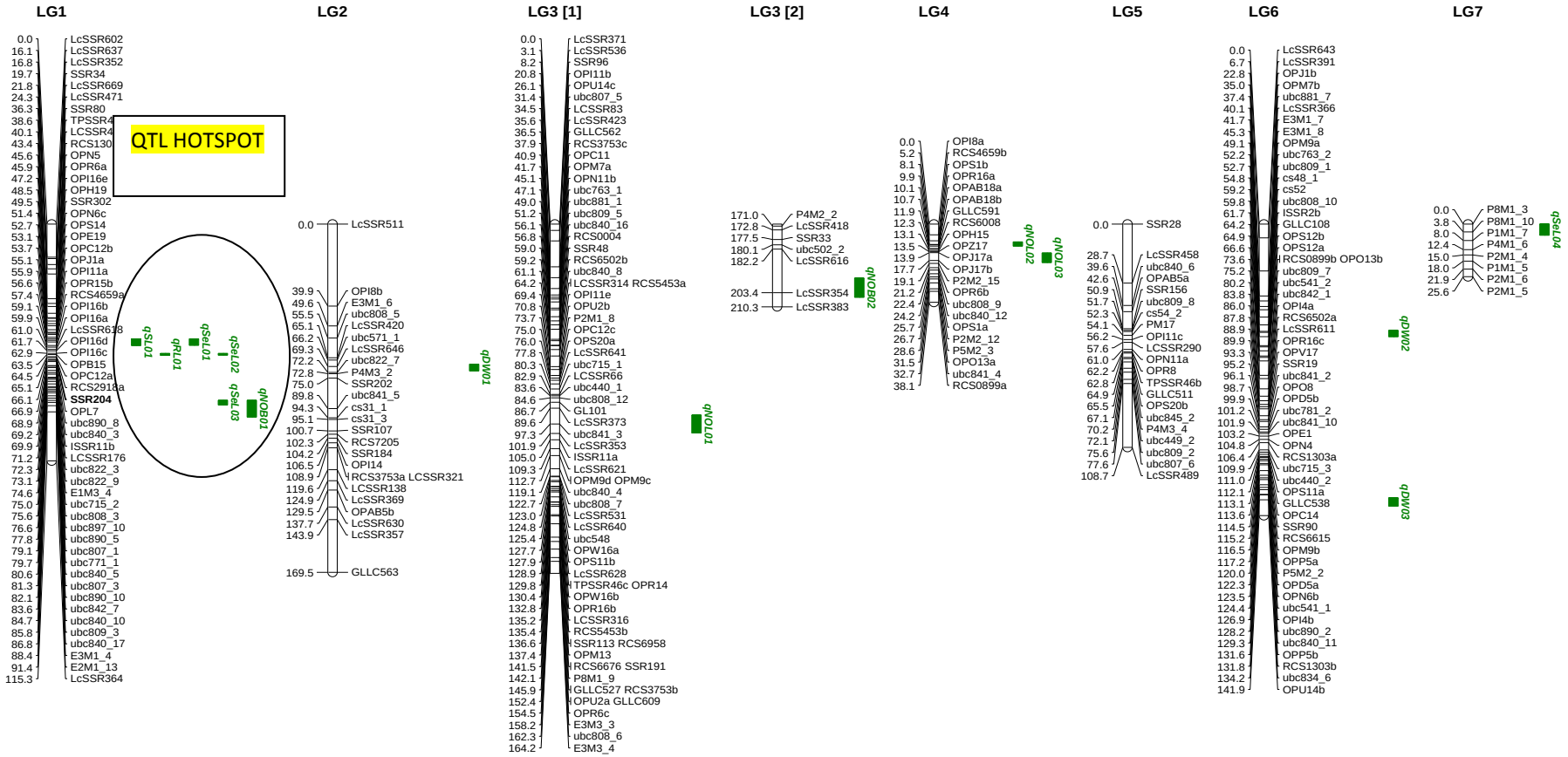


Fig 4.7. An intraspecific QTL linkage map of *L. culinaris* based on RIL mapping population generated by crossing *L. culinaris* cv. WA8649090 × *L. culinaris* cv. Precoz shows QTL HOT SPOT.

Future research

Future research work arising from this thesis should be directed towards the following areas:

a. Increasing the coverage of genome and marker density of the linkage map developed in this study would improve its usefulness by increasing the likelihood that markers will be tightly linked to genes of interest. This will also facilitate the alignment of different lentil genome maps and ultimately help in the development of a lentil consensus map.

b. In view of limited genetic variation in lentil, there is need to develop MAGIC (Multiparent advanced generation intercross) populations, which offer an alternative to traditional linkage or association mapping populations by increasing the precision of QTL mapping.

c. More recombinant inbred lines of lentil developed in the Department of Agricultural Biotechnology *viz*., WA 8649090 X Precoz, L 830 x ILWL 77 etc. which have been phenotyped for various traits could also be used for further validation of identified QTLs.

5. SUMMARY AND CONCLUSIONS

The present investigation entitled, "Identification of genomic regions controlling early plant vigour in lentil (*Lens culinaris* Medik.)" was undertaken to develop markers for construction of intraspecific map and to identify genomic regions linked with early plant vigour traits in lentil.

The study was carried out using recombinant inbred line (RIL) population of 94 individuals derived from an intraspecific cross of *Lens culinaris* ssp. *culinaris* (WA8649090 X Precoz) using single seed descent method. The RIL population was phenotyped for 6early plant vigour traits during 2017-2018 at Palampur.

Different types of SSR markers viz. *Lens* genomic SSRs (LcSSRs), red clover SSRs (RcSSRs),ISSRs and RAPD were used for parental polymorphism survey. Some of the already published SSRs (Hamweih et al. 2005;Phanet al. 2007; Saha et al. 2010b; Gupta et al. 2012) were used. A linkage map of lentil was constructed using an intra-specific RIL mapping population (*L. culinaris* cv. WA8649090 x *L. culinaris* cv. Precoz) using SSR markers. A total of 853 SSR, RAPD and ISSRs markers [433 (*Lens* genomic SSRs) + 145 (SSRs from other legumes viz. red clover) + 25 ISSR + 250 RAPD] were used to identify polymorphic primers between WA8649090 and Precoz, the parental lines of the mapping population. Of the 853 SSR primer pairs, 268 (31.41%) primer pairs were found to be polymorphic and were further used to genotype 94 individuals of the RIL mapping population for map construction. A total of 265markers were assigned map positions at LOD 4.0on seven linkage groups, spanning 809.4 cM distance of the lentil genome with an average marker density of 3.05 cM.

Further, the linkage map constructed was used for the identification of QTLs related to early plant vigour traits. Analysis of variance revealed significant differences for all the 6measured traits between the 'WA8649090' and the 'Precoz'.

The PCA of 94 RIL populations extracted two major principal components (eigenvalues > 1) that accounted collectively for 47.00 and 23.52 per cent of the

variance for early plant vigour trait, respectively. Principal component 1 explained 47 per cent of the data set variation, and was loaded positively with all measured traits. PC2 explained 23.52 per cent of the data set variation, and was positively loaded NOL and NOB traits only. Further, PCA showed that maximum contribution towards seedling length in the RILs followed by shoot length, root length, number of branches, dry weight and number of leaves.

A total of 14 QTLs (LOD ≥ 3) were detected across the environments. In total, 4 QTLs were detected for seedling trait, 3 for dry weight and number of leaves, 2 for number of branches traits, 1 each for shoot length and root length traits. LG1 contained total of 6 QTLs (three for seedling length and one each for shoot length, root length and number of branches); LG2 contained only one QTLs (one for dry weight) while LG3 contained of 2 QTLs (1 each for number of leaves and number of branches); LG4 had a total of 2 QTLs (both for number of leaves trait), LG5 not contained any of the QTLs; LG6 contained 2 QTLs (Each for dry weight trait) and LG7 contained only one QTLs (one for seedling length trait) .

Overall, one QTL cluster was identified which was located on LG1. QTL Cluster 1 contained QTLs for shoot length trait (SL 14.2 % PVE); Seedling length trait (qSeL0121.2 % PVE; qSeL02 19.4 % PVE); Root length traits (qRL01 13.2 % PVE) The region harbored 4 QTLs for three different traits explaining 13.2-21.2 % phenotypic variation. Thus, this cluster has been referred to as "*QTL-hotspot*" as this region contained several consistent QTLs with very high phenotypic variation explained and its introgression will definitely improve early plant vigour traits.

Evidently, the map generated in the present study although covered a significant portion of the genome, a further saturation of this map with additional markers (SSRs or SNPs) is imperative for its efficient utilization. With the long-term goal of understanding the genetic basis of early plant vigour traits, the present study was focused on identification of major QTLs for 6 traits in lentil. In conclusion, it is envisaged that the present linkage map, fortified with 265 SSR markers and 14 QTLs for early plant vigour traits would provide a means to breeders for further use in genetic enhancement of this crop species. However, a denser genetic linkage maps with large number of markers with the inclusion of SNPs would facilitate the

identification of more resolved and fine QTL positions which can significantly improve the resolution of identified QTLs for mapping. The identification of QTLs controlling agronomically important traits would improve our genetic understanding of these traits and finally provide the basis for MAS of these traits. Therefore, QTL validation and fine mapping is the next step towards successful application of these findings in MAS.

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APPENDICES

Appendix-I: Mean values of traits studied:

Genotypes	SL (cm)	RL (cm)	SeL (cm)	DW (mg)	NOL	NOB
1	3.2	1	1.55	0.01	113	3.5
2	4.5	3	7.35	0.03	123.5	7.5
3	2.8	1.5	4.25	0.01	112.5	6
4	4.8	1.2	7.8	0.02	112.5	8.5
5	3	0.3	3.92	0	117	4.5
6	3.1	2.1	5.65	0.02	129	5
7	4.2	3.5	7.9	0.04	134.5	7.5
8	2	3.5	5.75	0	136.5	3
9	3.6	0.7	4.05	0.01	128.5	7.5
10	4.37	1.42	5.3	0.02	127.5	7
11	4.11	2.97	6.69	0.03	128.4	7.43
12	3.28	2.25	5.3	0.02	128.5	5.5
13	2.9	3.9	6.25	0.02	120.5	8.5
14	5.8	2.9	8.1	0.03	135.5	5.5
15	2.1	3.4	6.15	0	109	5
16	1.5	1.4	2.85	0.02	133.5	9.5
17	5.8	2.4	8.27	0.03	139.5	7.5
18	4.2	3.5	6.15	0.04	119	5.5
19	6.39	3.2	9.06	0.03	131	8.5
20	7.9	3.1	17.6	0.06	111	7
21	2	2.3	4.25	0.02	107.5	7.5
22	5.53	2.86	7.99	0.04	107	5
23	6.57	2.4	9.02	0.04	126	8.5
24	3.3	5.2	8.6	0	113.5	5.5
25	9.08	3.13	12.1	0.08	138.5	8
26	5.8	2.2	6.8	0.04	115.5	5.5
27	5.6	2.9	7.45	0.06	136	12.5
28	5.2	2.6	6.45	0.05	133	10.5
29	4.9	2.7	5.35	0.04	133	11
30	3.9	2.3	5.9	0.04	134.5	8
31	7.44	2.37	9.13	0.06	123.5	9.5
32	6.68	3.88	9.8	0.05	137.5	13.5
33	7.08	5.26	11.81	0.06	150	17.5
34	4.3	1.6	5.85	0.03	137	14.5
35	3.1	2.7	5.58	0.02	124.5	9.5
36	7.02	4.16	10.66	0.06	126	8
37	3.9	2.5	6.55	0.02	132.5	7.5
38	5.9	2.3	7.78	0.05	126	7.5
39	3.1	2.3	5.05	0	118.5	5.5
40	3.4	1.2	4.55	0.02	143.5	6.5
41	4.1	1.3	5.35	0.02	136	7.5
42	2.5	1	3.15	0.01	126.5	6.5
43	3.5	2.1	4.35	0	127.5	7.5
44	2.1	1.2	3.05	0.02	111.5	5

45	7.08	3.05	10.14	0.05	143.5	10.5
46	1.6	1.2	2.9	0.01	125.5	6.5
47	7.2	5.8	12.8	0.06	126	7.5
48	1.55	1.95	3.15	0.01	136	6.5
49	4.1	1.45	5.13	0.02	133.5	7.5
50	1.9	3	4.25	0.01	130	7.5
51	6.6	3.4	10	0.04	140.5	8
52	5.4	1.2	6.95	0.05	138	8.5
53	3.6	1.45	5	0.02	124.5	6
54	6.43	3.2	8.87	0.04	127	7
55	4.38	3.28	7.21	0.02	144.5	11.5
56	5.89	5.65	11.15	0.04	147.5	11.5
57	4.4	1.73	5.36	0.02	143.5	12
58	6.3	2.6	7.99	0.04	126.5	6
59	4.23	3.2	7.42	0.02	126.5	8.5
60	4.8	3.35	7.58	0.02	116.5	6
61	3.8	3.4	7.15	0.02	143.5	9.5
62	2.1	1.5	4.23	0.01	146	10.5
63	3.7	1.6	4.35	0.02	124	6
64	3.1	2.75	4.65	0.01	140.5	10
65	3.3	2	4.97	0.02	126.5	5.5
66	4.7	4.95	9.18	0.03	145	10.5
67	2.1	3.4	4.65	0.04	117	3.5
68	5.1	8.9	10.45	0.14	137.5	10.5
69	2.3	3.6	6.25	0.05	117	5.5
70	4.8	6.5	9.25	0.1	136	8.5
71	3.1	4.6	6.35	0.06	134	7
72	4.83	3.63	8.01	0.06	125.5	7.5
73	4.7	5.08	9.13	0.03	137	9.5
74	3.1	5.1	6.25	0.04	131.5	7
75	2.1	4.4	7.55	0.05	112	5
76	5	3.6	8.3	0.03	123.5	7
77	3.3	4	10.3	0.02	131	6.5
78	2.4	3	5.2	0.01	117.5	2.5
79	1.6	2	3.55	0.01	127	5.5
80	2.3	3.5	4.75	0.02	127.5	6.5
81	4.11	2.97	6.69	0.03	128.4	7.43
82	4.2	5.9	10.05	0.05	132.5	7.5
83	1.8	2.35	3.45	0	112.5	3.5
84	2.3	2.62	4.15	0.01	127.5	3.5
85	3.2	3.5	6.35	0.11	126.5	7.5
86	2.3	3.5	4.25	0.02	125.5	5
87	4.63	3.8	8.17	0.12	97	2.5
88	4	3.8	6.42	0.02	134	8.5
89	4.1	3.9	5.88	0.02	126	6
90	4.4	4.7	8.05	0.11	124.5	6.5
91	3.2	2.4	4.35	0.03	121.5	6.5
92	3.8	2.4	6.15	0.21	122.5	6
93	2.9	1.9	4.05	0.03	155	6.5
94	3.2	2.4	4.25	0.03	151.5	9

Appendix-II: RAPD and ISSR primers used

S.No.	Primer Name	Primer Sequence	No. of Bases
1	OPA10	GTGATCGCAG	10
2	OPAB05	CCCGAAGCGA	10
3	OPAB06	GTGGCTTGGA	10
4	OPAB18	CTGGCGTGTC	10
5	OPB15	GGAGGGTGTT	10
6	OPC12	TGTCATCCCC	10
7	OPC14	TGCGTGCTTG	10
8	OPD05	TGAGCGGACA	10
9	OPE01	CCAAGGTCCC	10
10	OPE17	CTACTGCCGT	10
11	OPE19	AACGGTGACC	10
12	OPH15	AATGGCGCAG	10
13	OPH19	CTGACCAGCC	10
14	OPI 8	TTTGCCCGGT	10
15	OPI11	ACATGCCGTG	10
16	OPI14	TGACGGCGGT	10
17	OPI16	TCTCCGCCTT	10
18	OPI4	ACGGATCCTG	10
19	OPJ01	CCCGGCATAA	10
20	OPJ17	ACGCCAGTTC	10
21	OPL07	AGGCGGGAAC	10
22	OPM07	CCGTGACTCA	10
23	OPM09	GTCTTGCGGA	10
24	OPM13	GGTGGTCAAG	10
25	OPM17	TCAGTCCGGG	10
26	OPM18	CACCATCCGT	10
27	OPN04	GACCGACCCA	10
28	OPN05	ACTGAACGCC	10
29	OPN06	GAGACGCACA	10
30	OPN11	TCGCCGCAAA	10
31	OPN19	GTCCGTACTG	10
32	OPO 08	GCTCCAGTGT	10
33	OPO 13	GTCAGAGTCC	10
34	OPP05	CCCCGGTAAC	10
35	OPR06	GTCTACGGCA	10
36	OPR08	CCCGTTGCCT	10
37	OPR14	CAGGATTCCC	10
38	OPR15	GGACAACGAG	10
39	OPR16	CTCTGCGCGT	10
40	OPS01	CTACTGCGCT	10
41	OPS11	AGTCGGGTGG	10
42	OPS12	CTGGGTGAGT	10
43	OPS14	AAAGGGGTCC	10
44	OPS20	TCTGGACGGA	10
45	OPU02	CTGAGGTCTC	10
46	OPU14	TGGGTCCCTC	10
47	OPV17	ACCGGCTTGT	10
48	OPV20	CAGCATGGTC	10
49	OPW03	GTCCGGAGTG	10
50	OPW15	ACACCGGAAC	10
51	OPW15	ACACCGGAAC	10
52	OPW16	CAGCCTACCA	10
53	OPZ17	CCTTCCCACT	10
54	ISSR02	ACTGACTGACTGACTG	16
55	ISSR 11	AGAGAGAGAGAGAGAGAGAGC	21
56	ISSR 21	GTGGTGGTGGTGGTGGTGA	19

Appendix-III: Lentil SSRs (Hamwieh et al. 2005, 2009) used in the study

S.No	Primer Name	Forward Sequence	Number of bases	Reverse Sequence	Number of bases	Annealing Temperature (Tm) used for PCR (^{0}C)	Expected size (bp)	Microsatellite motif
1	SSR 19	GACTCATACTTTGTTCTTAGCAG	23	GAACGGAGCGGTCACATTAG	20	58	250	$(TG)_{14}$
2	SSR 48	CATGGTGGAATAGTGATGGC	20	CTCCATACACCACTCATTCAC	21	57	165	$(TG)_{13}$
3	SSR 90	CCGTGTACACCCCTAC	16	CGTCTTAAAGAGAGTGACAC	20	55	181	$(TG)_{11}(AG)_{10}$
4	SSR 113	CCGTAAGAATTAGGTGTC	18	GGAAAATAGGGTGGAAAG	18	51	211	$(AC)_{17}(AT)_{13}$
5	SSR 183	GCTCGCATTGGTGAAAC	17	CATATATAGCAGACCGTG	18	52	119	$(AC)_8$
6	SSR 184	GTGTGTACCTAAAGCCTTG	19	GTAAGTTGATCAAACGCCC	19	55	250	$(GT)_{10}(AT)_{15}(GT)_{19}$
7	SSR191	GCAAATTTCTTGGTCTACAC	20	GGGCACAGATTCATAAGG	18	53	238	$(AC)_{17}(AT)_{13}$
8	SSR 204	CACGACTATCCCACTTG	17	CTTACTTTCTTAGTGCTATTAC	22	53	186	$(TG)_4 + (AC)^b{}_7$
9	SSR207	GAGAGATACGTCAGAGTAG	19	GATTGTGCTTCGGTGGTTC	19	55	227	$(CA)_{22}$
10	SSR 302	CAAGCCACCCATACACC	17	GGGCATTAAGTGTGCTGG	18	56	261	$(TA)_{15}(CA)_{11}$
11	SSR 309-2	GTATGTCGTTAACTGTCGTG	20	GAGGAAGGAAGTATTCGTC	19	50	182	$(AT)_3GT(TA)_3T(TAT)_6$
12	SSR 317-1	GTGGGTGTAATTATTGCTAC	20	GTATCAAACTTATGGTGAAATC	22	53	308	$(AT)_4(GT)_{16}(GC)_6GTGGC(GT)_5A(TG)_8 + (TAA)_5$

Appendix-IV: Lentil SSRs (Saha et al. 2010b) used in the study

S.No	PrimerName	Forward Sequence	Reverse Sequence	Number of bases	AnnealingTemperature (Tm) used for PCR (^{0}C)
1	GLLC 511	ATTGAGAGGAGGCGGAGAA	CGCGTGTCTCTCTCTCTCAC	39	55
2	GLLC 527	GTGGGACGGTTTGAATTTGA	GAACATAAAATGGGAGTGTCACAA	44	55
3	GLLC 562	TGTGTAGGCACATCAACAAAA	GGTGGGCATGAGAGGTGTTA	41	55
4	GLLC 591	TGTTTGATGTACCTCAGGCTTA	TGAACTGATGAGGAGGACGTT	43	55
5	GLLC 614	AACCCCAGCCAGATCTTACA	AAGGGTGGTTTTGGTCCTATG	41	55

Appendix-V: LcSSRs (Sabhyata Bhatia, NIPGR, New Delhi) used in developing linkage map

S.No	Primer Name	Forward Sequence	Reverse Sequence	Annealing Temperature (Tm) used for PCR (^{0}C)
1	LcSSR66	Not available	Not available	Touch down
2	LcSSR70	Not available	Not available	Touch down
3	LcSSR83	Not available	Not available	Touch down
4	LcSSR112	Not available	Not available	Touch down
5	LcSSR138	AATCGTTGCAAGTAGGAAAAGG	AGGGGTTCATCTAAGGGGATAA	Touch down
6	LcSSR176	CCCCTATTATCATTATTGGTGCT	AGGGAAATCCTCACTACCCATT	Touch down
7	LcSSR 198	CTCAAGGAACAAACCAGCAATA	CGAGACCATTCTAAACACACGA	Touch down
8	LcSSR290	TTTGCATAGTGACAGAAGATCCA	AGCATCATCACTGCCATCAT	Touch down
9	LcSSR314	Not available	Not available	Touch down
10	LcSSR316	Not available	Not available	Touch down
11	LcSSR321	Not available	Not available	Touch down

Appendix-VI: Cross genera SSRs of *Trifolium pratense* (Sato et al,2005) used in developing linkage map

S. No	Primer name	Forward sequence	Reverse sequence	Annealing temperature
1	RCS2918	CGCCAATTTCATTGTTGGAT	GAGGTTTGGAGTGTTGCCAT	50
2	RCS5453	Not available	Not available	53
3	RCS7205	TATCACACCCCATTTGGCTT	ATGATGGCAACGGTGGTAA	53
4	RCS1303	CAGCAATCCAACGTTTCTGA	ATCATCACCAGCTTCAGCAC	53
5	TPSSR45	TGTGTTATGGTGAAGTTCAAAATATAATTTC	CCAATGGCGTCAATGGTCTC	53
6	TPSSR46	TCAAATAAAACTTTCATAACGTTCATCTC	TCCGAAGAAACCATTATCTACGTTG	50
7	RCS3753	ACATTTCCGTTGAAGGCAAG	ATCAGGTGGATGGCATGATT	53
8	RCS6615	TCCAATTTCTCCACTCTCACA	GATATCAGGGTGGCATCGTT	50
9	RCS6958	TCAACAAAACACAAACCACTCTC	AGGGAGAAATGCCATGTGAG	50
10	RCS6502	TCACAATGTCACCAACAAAACA	TTGCACTTTTTCTCAGTGGCT	53
11	RCS6008	CGCAAACCTCTTCCTCTCTC	TCGGTGGATCCTTCAACTTC	53
12	RCS6676	CTCTTTCACCGCCTTGAGAC	TGGTGGAGTTGTTTTGGTGA	53
13	RCS0899	CAAACAGGGTTTGTTGCTGA	TGTTGTCAGGTCAAAGACTCAAA	50

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Thesis Title in M.Sc. –Identification of genomic regions controlling early plant vigour in lentil (*Lens culinaris* Medik.).