GENETIC AND PHENOTYPIC ASSESMENT OF IRON AND FOLATE CONCENTRATION IN LENTIL (*LENS CULINARIS* MEDIK.)

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Micronutrients and vitamins are chemical elements required in trace quantities for normal human growth and development. Micronutrients and vitamin deficiency is prevalent throughout the world. The first objective of this research was to determine folate concentration in 10 lentil genotypes and evaluate the effect of environment on folate concentration. Folate concentration ranged from 216 to 290 µg/100 g with a mean of 255 µg/100 g and the concentration differed across years and locations. A significant genotype × environment interaction effect was observed for lentil folate concentration. The second objective was to measure the iron, zinc, copper, calcium and magnesium concentration in 26 cultivated and wild lentils. Significant variation in Fe, Zn, Cu, Ca, and Mg concentration among *Lens* species and no single genotype had high concentrations of all micronutrients.The third objective was to determine genetic diversity among 29 cultivated and wild lentils using 39 simple sequence repeat markers. Thirteen of 39 SSR markers were polymorphic among the 29 lentil genotypes. Cluster analysis grouped the genotypes into 4 clusters broadly based on the genotyping data and this grouping had correspondence with the pedigree relationships of the genotypes. The fourth objective was to develop expressed sequence tags-simple sequence repeats (EST-SSRs) markers in lentil. Lentil EST sequences (9513) from the NCBI database were assembled into 4053 unigenes. Unigenes were screened for simple sequence repeats and 348 primer pairs were designed. Fifty-seven primer pairs were polymorphic among the 22 lentil genotypes providing additional gene-specific primers for use in lentil breeding. The fifth objective was to develop gene specific molecular markers for iron metabolism related genes in lentil and to study their gene expression in the presence of excess iron. Gene specific markers were developed for *Ferritin-1, BHLH-1,* and *IRT-1* to allow detailed study of the iron metabolic pathway in lentil. Differential gene expression of

Ferritin-1 and *IRT-1* under excess iron was observed at 2 hours but not at 8 hours and 24 hours. Results of these studies contribute to a broad understanding of the genetic variation, environmental influence on and expression of genes related to micronutrient and vitamin concentration and metabolism in lentil.

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DEDICATION

I dedicate this piece of work to my grandfather, late Mr. Satyendra Nath Sengupta, who ignited that little fire within me during the very early stage of my academic career about the importance of education in life. I also want to dedicate this thesis to the lentil growers' community worldwide.

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LIST OF ABBREVIATIONS

AFLP……Amplified Length Polymorphism

BHLH……...Basic Helix Loop Helix

BMI……..Body Mass Index

CDC…….US Centers for Disease Control and Prevention

DNA…….Deoxyribonucleic Acid

EST-SSRs…….Expressed Sequence Tag-Simple Sequence Repeats

FAO……..Food and Agriculture Organization

FIT……Fer-like Fe Deficiency Induced Transcription Factor

GO……Gene Ontology

ICARDA……...International Center for Agricultural Research in the Dry Areas

IPS…….Inter Pro Scan

IRT……....Iron Transporter Like Protein

MA……Mugineic Acid

MIRA…… ...Mimicking Intelligent Read Assembly

MISA…….Microsatellite Identification Tool

NCBI…….National Center for Biological Information

NTDs…….Neural Tube Defects

PIC…….Polymorphism Information Content

qPCR……..Quantitative PCR

QPM……...Quality Protein Maize

QTL……Quantitative Trait Loci

RDA……....Recommended Daily Allowance

RIL……..Recombinant Inbred Line

RP-HPLC……Reverse Phase-High Performance Liquid Chromatography

SCN……Standing Committee on Nutrition

SNP……Single Nucleotide Polymorphism

SSRs…….....Simple Sequence Repeats

THF……Tetrahydrofolic Acid

UN…….....United Nations

UNICEF…….United Nations Children's Fund

UPGMA…….Unweighted Pair Group Method With Arithmatic Mean

UPLC-MS……..Ultra Performance Liquid Chromatography-Mass Spectrometry

USDA/ARS……United States Department of Agriculture/Agriculture Research Service

VIT…….....Vacuolar Iron Transporter

WHO……..World Health Organization

ZIP……..Zinc and Iron Transporter Like Protein

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CHAPTER 1. GENERAL INTRODUCTION

More than 20 million childhood deaths occurr every year due to micronutrient deficiency (Anonymous 2008), and diet-related non-communicable diseases (cardiovascular diseases, cancers, chronic respiratory diseases and diabetes) (Bouis and Welch 2010). The United Nations (UN) recently announced that the increase in chronic, non-communicable diseases including diabetes, heart disease, and cancer has resulted in 36 million deaths around the world annually, claiming more lives than all other causes combined (World Health Organization, WHO 2005). These chronic diseases are not isolated to developed countries and are even more pronounced in the developing world. Such chronic diseases have caused more deaths than infectious diseases throughout the world (except Africa) in recent years (UN 2011). Therefore, enrichment of micronutrients in staple food crops is important for nutrition security of human beings especially in the developing world.

Anaemia is a common nutritional disorder affecting humans and according to the World Health Organization (WHO 2005), about two billion people are anemic. Anaemia is a health condition characterized by low hemoglobin concentrations in the blood and threshold levels vary based on gender and race. There are generally two causes for anaemia: dietary micronutrient and vitamin deficiency like Fe, folate, vitamin B12 and or Vitamin A deficiency and infectious diseases such as malaria, hookworm infections, schistosomiasis, and thalassaemia (WHO 2007). Iron deficiency may or may not be accompanied with anaemia but always has an important negative impact on human health. The effect of Fe deficiency is more pronounced in pregnant women and children (WHO 2007). Sometimes this has severe consequences leading to mortality of the new born children or even the fetus during the prenatal stage.

Table 1.1. Prevalence of micronutrient deficiency in the world.

Deficiency	Affected people	Health consequences
Iron (Fe)	2 billion	Reduced cognitive ability, anaemia, maternal mortality (UN system standing committee on nutrition 2004)
Vitamin A	250 million	Night blindness, xerophthalmia, keratomalacia and immune system failure (UN system standing committee on nutrition 2004)
Zinc (Zn)	2 billion	Infectious diseases, poor child growth, maternal mortality, reduced birth weight (WHO 2005)

Children with acute iron deficiency show mental retardedness, laziness and in the case of working persons, reduced capacity to work (WHO 2007).

Folic acid (synthetic oxidized form of naturally occurring folates) consists of a p-aminobenzoic molecule linked to a pteridine ring and one molecule of glutamic acid. Food folates, which exist in various forms, contain additional glutamate residues, making them polyglutamates (Bailey and Gregory 2006). Folate is a water soluble B vitamin involved in numerous biochemical reactions involving one carbon transfer, for example, purine and pyrimidine synthesis as well as amino acid interconversions (Krumdieck 1990). Prevention of chromosome breakage and hypomethylation of DNA (Fenech 2001) by folates aids in the reduction of risk factors leading to cancer and also plays a critical role in regulating homocysteine status, an important risk factor for cardiovascular ailments (Pancharuniti et al. 1994). Lower levels of plasma folate are correlated with various health risks including neural tube defects (NTDs), which are a prime concern along with a few other congenital defects (Berry et al. 2000). Folate deficiency is also associated with macrocytic anaemia (enlarged red blood cells) (Boushey et al. 1995). There were reports of strong correlation between folate deficiency and iron deficiency causing anaemia (WHO 2007).

Populations in developing countries including Southeast Asia and Africa consume mostly cereal based diets. The 'Green Revolution' contributed to sustaining the cereal based diet to avert famine (Bouis and Welch 2010). The cereal based diets in most cases supply adequate calories but are insufficient to provide recommended quantities of micronutrients to the human body. Many countries have observed an increase in malnutrition cases due to the dependence on cereal based cropping systems (Welch and Graham 1999).

Biofortification is the development of micronutrient and or vitamin rich crops using traditional crop improvement practices as well as modern biotechnology tools. It is a more sustainable and cost effective method than food supplementation, fortification and diet diversification. Though agronomic biofortification i.e. application of micronutrients through soil amendments, foliar sprays or irrigation water is practiced, in the case of Fe has not been successful (Bouis and Welch 2010; Tagliavini et al. 2000; Tagliavini and Rombola 2001). Genetic biofortification is a cost effective way to provide access to nutritional foods for people who are living in remote, less privileged areas of the world as it requires a one-time initial investment and easy seed multiplication through plant breeding interventions make it a promising approach.

Under the umbrella of the HarvestPlus program of CGIAR (Consultative Group of International Agricultural Research) the initial phase of biofortification programs included six food crops, common bean, cassava, maize, rice, sweet potato, and wheat. The initial phase of investment resulted in many success stories like orange sweet potato (OSP) cultivars with high levels of β –carotene (over 200 mg/g) (Bouis and Islam 2012), and beans with improved agronomic traits and grain type and 50–70% more Fe have been bred through conventional breeding (Nestel et al. 2006). Though conventional breeding is still the focus of the HarvestPlus

program, research into transgenic approaches are in some cases necessary and are being used. The most popular and earliest example of a success story of transgenic biofortification research was development of Golden Rice or β -carotene rich rice. Golden rice transgenic lines have been tested in field or controlled trials in the Philippines (transgenic of RC-28), and Bangladesh (transgenic of BRRI Dhan-29) and will certainly help to fight against Fe deficiency. Recently, an Fe rich, high yielding pearl millet cultivar, ICTP 8203-Fe, has been launched as a result of collaborative effort between the HarvestPlus program and Nirmal Seeds, a Hyderabad, India based seed company. The ongoing HarvestPlus phase included more food crops and a few more food legumes, especially lentil which is a regular component of the daily diet in major regions in South and Southeast Asia. Food legumes play a significant role as far as food security by supplying protein, dietary fiber along with essential micronutrients like Fe, Zn and Se with beta carotene and folates.

Iron and folate bioavailability of a staple food crop mainly depends on food matrix factors. The concentration of promoter and inhibitor compounds in any food crop is influenced by both genetic and environmental factors. Modern plant breeding and molecular biology tools now make it possible to reduce antinutrients, such as phytic acid (PA) or increase the concentration of promoter substances, such as beta-carotene, ascorbic acid and phytoferritin in plant foods. Promoters and inhibitors of Fe absorption within the food matrix must be considered with respect to the bioavailability of non-heme Fe in a food crop (Cook et al. 1972). Phytic acid (PA), nearly ubiquitous in plants and used as the primary form of phosphorous (P) storage, inhibits absorption of Fe in the gut (Turnbull et al. 1962). Other inhibitors include fiber, heavy metals, and certain polyphenols and tannins (Glahn et al. 2002).

Enrichment with prebiotics, beta-carotene, ascorbic acid and phytoferritin in plant-based diets has been shown to enhance the bioavailability of non-heme Fe in human (Welch 2002). Prebiotics improve Fe bioavailability as a result of biological fermentation of short chain polymers by natural microflora present in the colon (Yeung et al. 2005). Addition of vitamin A or beta-carotene can improve Fe bioavailability from plant-based foods (e.g., rice, wheat, corn) (Garcia-Carsal et al. 2000).

Analysis of lentil food matrix components, along with cell culture and preliminary human nutrition studies, reveals clear mineral absorption promoter and inhibitor roles in modulating the levels of mineral bioavailability. Lentils contain high levels of Fe absorption promoters, such as prebiotics and beta-carotene, and are low in antinutrients, such as phytic acid and polyphenols (Thavarajah and Thavarajah 2012). It is reported that molar ratios of phytic acid:Fe above 10 lead to reduced human Fe bioavailability (Ariza-Nieto et al. 2007).

The term ‘folates’ collectively denotes the naturally occurring derivatives of folic acid (vitamin B9). Among the many naturally occurring polyglutamyl forms of tetrahydrofolic acid (THF) 5-methyl-THF, 10-formyl-THF, and 5-formyl THF, are the predominant forms of storage in food legumes (Yarbaeva et al. 2011; Hefni et al. 2009). Microbiological assay of folate estimation is erroneous (Hefni et al. 2009). It usually gives higher values compared to more accurate HPLC methods using the tri-enzyme extraction method (Talamond et al. 2000).

Lentil (*Lens culinaris* ssp. *culinaris* Medik.) is an important cool season food legume crop cultivated throughout West Asia, North Africa, the Indian subcontinent, North America, and Australia (FAOSTATS 2010). Global production of lentil is around 5 MT with 392 KT being produced in the United States; 1947 KT from Canada (the largest exporter), 900 KT from India (the largest consumer and importer), 711 KT from Bangladesh, 447 KT from Turkey, 140 KT

from Australia, 125 KT from China, 123 KT from Ethiopia, 79 KT from Iran, and 77 KT from Syria (FAOSTAT 2010). Lentil is rich in protein (20-30%) with dietary fiber and a wide range of micronutrients. Lentil has a capacity to fix biological nitrogen making it a useful component for soil fertility and water management in cereal based cropping systems.

Genetic variation exists for micronutrient concentration (Graham and Welch 2000; Bouis 2003; Graham et al. 2001) and plant breeding tools can improve β-carotene, iron, zinc, and other minerals in food crops through selection for appropriate genetic material (Nestel et al. 2006). Micronutrient density and yield are positively correlated unlike protein content and yield (Nestel et al. 2006). Also, it is possible to combine multiple nutrition traits in a single cultivar along with high yield (Nestel et al. 2006).

Knowledge of genetic diversity for a particular trait of interest can be a predictive tool for estimating genetic variation in segregating populations or hybrid progeny. Development of molecular markers linked with the loci controlling micronutrient concentration requires initial large scale evaluation of available germplasm sets of different food legumes.

Table 1.2. Food matrix factors acting as a promoter or inhibitor to Fe bioavailability in lentil.

Food matrix factor
Promoters
1. Prebiotics: inulin and fructans
2. Beta-carotene
3. Organic acids: ascorbic acid
4. Amino acids
Inhibitors
1. Phytic acid
2. Fiber
3. Haemagglutinins
4. Phenolics
5. Heavy metals

Source: Welch 2002.

After identification of potential genotypes, suitable mapping populations can be developed for particular traits (Talukder et al. 2010; Beebe et al. 2000). Studies conducted to map and tag the gene(s)/QTL controlling micronutrient status in legumes and model plants have reported quantitative inheritance patterns resulting in identification of gene(s)/QTL(s) capable of explaining modest amounts of phenotypic variation for micronutrient concentration [Sompong et al. 2012 (for phytic acid in mungbean), Blair et al. 2005; Gelin et al. 2007; Cichy et al. 2009; Blair et al. 2010 a,b; (for Fe and Zn in common bean), Sankaran et al. 2009 (for several mineral elements in *Medicago truncatula*), Waters and Grusak 2008 (for several seed mineral contents in *Arabidopsis thaliana*), Walker et al. 2006 (for phytic acid in soybean)].

The objectives of this research were:

1. To quantify the folate concentration of lentil genotypes and characterize the genotype x environment interaction effect on folate concentration.
2. To determine iron and zinc concentration among cultivated and wild genotypes of lentil (*Lens culinaris* Medik.).
3. To measure genetic diversity of a set of cultivated and wild lentil genotypes with simple sequence repeats (SSRs) markers.
4. To develop expressed sequence tagged-simple sequence repeats markers (EST-SSRs) in lentil and validate those markers within a diverse set of lentil genotypes.
5. To develop useful molecular markers in lentil for iron metabolism related genes and evaluate their differential expression under excess iron.

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CHAPTER 2. LITERATURE REVIEW

Malnutrition

"Status of child undernutrition remains unacceptable throughout the world, with 90 percent of the developing world's chronically undernourished children living in Asia and Africa" (UNICEF 2009). Children less than five years of age suffer more from malnutrition (UNICEF 2009). In developing countries like India, 43 percent of children under the age of five are underweight and 48 percent are stunted (Arnold et al. 2009). Malnutrition is more common for children of mothers who are undernourished themselves than for children whose mothers are not undernourished (UNICEF 2009). In 2013 about 17,000 child deaths occurred each day (WHO 2015b) and about 35% of annual child deaths under the age of five were due to malnutrition (WHO 2013).

The term malnutrition refers to both undernutrition and overnutrition. Hence, obesity or overweight is also referred to as malnutrition. Overweight and obesity both indicate the excessive accumulation of body fat. Body mass index (BMI) is a ratio between body weight in kilograms (kg) and the square of body height in meters (m^2). BMI equal or above 25 is considered overweight and BMI equal to or above 30 is considered obese. Obesity is a serious issue in developed countries and worldwide where 1.9 billion are overweight adults (18 years old or above), and at least 600 million are obese (WHO 2015a).

Intake of high carbohydrate, high fat food (energy-dense food) along with minimal or no physical activity increases the frequency of overweight or obese children as well as adults in all age groups. China and some African nations have the lowest percentage of obese indivduals (WHO 2015a). It was estimated that 42 million children under the age of five were overweight or obese in 2013 (WHO 2015a). Malnutrition initially included calorie and protein insufficiency as

sole parameters to be considered, however, today, micronutrient and vitamin deficiency are also being considered as components of malnutrition. There is opportunity to reduce malnutrition by ensuring proper nutrition of children in the first two years of life (1000 days), girls during adolescence, and mothers during pregnancy and lactation. Vitamin and micronutrient deficiencies are highly prevalent throughout the developing world. Anaemia in young children is a serious concern, because it may increase the chances of getting infected by infectious diseases and generally leads to impaired growth and development (UNICEF 2009).

Micronutrients and vitamins

Micronutrients and vitamins are chemical compounds important to human nutrition. At least 30 essential micronutrients exist that cannot be synthesized by the human body and must be obtained through food, either of plant or animal origin (Shergill-Bonner 2013). Recommended dietary intake varies by age, sex, and special circumstances and many countries adopt nutritional standards set forth by international organizations like FAO (Food and Agriculture Organization) and WHO (World Health Organization). United States of America and European Union have their own standard recommendation for daily intake of micronutrients and vitamins.

Micronutrients are required in trace quantities and recommended daily allowances are measured in milligrams per day and they act as cofactors in metabolic pathways. For example, zinc is a cofactor in hundreds of enzymes (Shergill-Bonner 2013). Vitamins are also required in trace quantities and recommended daily allowances are measured in micrograms. Vitamins and their derivatives function as coenzymes for biochemical reactions.

Iron

Iron (Fe) is an important micronutrient for plant growth and survival and must be supplied from the soil solution. Fe is involved in numerous biological or cellular functions including photosynthesis, respiration and other redox reactions (Kim and Guerinot 2007). Iron is one of the most abundant elements on earth, however, it is not readily available to plants due its low solubility in the soil solution (Guerinot and Yi 1994). Deficiency in Fe supply to the plants results in development of deficiency symptoms including veinal or inter-veinal chloroloris, stunting, changes in color of the leaves and other green plant organs. Accumulation of Fe in high concentrations is toxic to the plants and the optimum concentration ranges from 10^{-9} to 10^{-4} M for optimum plant growth depending on the plant species. During respiration reduction of molecular oxygen produces superoxides and peroxides. Superoxides and peroxides are catalyzed by iron ions to generate hydroxy radicals (Halliwell and Gutteridge 1992). Plants have evolved systems to avoid any cellular damages arising from free radicals and to maintain iron homeostasis, an equilibrium state between iron deficiency and iron toxicity.

There are two strategies within plants for Fe uptake and transport from the soil solution. Strategy I is reduction based and is common for dicot species and involves the plants extruding protons and phenolic compounds into the rhizosphere (the volume of soil area where roots are spread out) to lower the soil pH and make Fe^{+3} more soluble (Olsen et al. 1981). Strategy II is used by monocots and is chelation-based where plants release mugineic acid (MA) phytosiderophores which bind to Fe^{+3}. Phytosiderophores are Fe^{+3} solubilizing molecules secreted by graminaceous plants (plant family: poaceae) under Fe deficient conditions (Takagi et al. 1976). Nine different types of MAs have been identified and all are synthesized from a

common precursor, S-adenosyl-methionine (Bashir et al. 2006). Strategy II is considered more efficient for Fe uptake and transport.

Fe is bound to chelating compounds as it reaches the root symplast and is then released into the xylem. Fe release from the xylem vessel to leaf tissues is not clearly understood (Kim and Guerinot 2007). A small proportion of Fe is also transported through phloem sap and it is believed that many ZIP (zinc and iron transporter like protein) and IRT (iron regulating transporter like protein) genes facilitate movement of Fe across membranes in leaf and shoot tissues (Vert et al. 2002).

Vacuoles accumulate Fe and in *Arabidopsis*, VIT1 (Vacuolar Iron Transporter I) is an important transporter responsible for Fe storage (Kim et al. 2006). In addition to VIT, the Nramp gene family (Natural Resistance Associated Macrophage Proteins) is active in transport of Fe (Curie et al. 2001). Ferritin proteins are the principal form of Fe storage in plants. It can store up to 4500 atoms of Fe per molecule. Ferritin is controlled by a gene family and ferritin coding genes and proteins share sequence similarity across plant and animal species and genera which indicates conservation of function. Many different ferritin genes have been cloned and characterized in different plant species including *Arabidopsis* (Petit et al. 2001), maize (Fobis-loisy et al. 1995), *Medicago truncatula* (Györgyey et al. 2000), soybean (*Glycine max*) (Masuda et al. 2001), common bean (*Phaseolus vulgaris*) (Spence et al. 1991) and rice (Lucca et al. 2001). A limited number of species use both strategies for Fe uptake and translocation, for example, rice (*Oryza sativa*) (Inoue et al. 2009). Translocation through the casparian strip is mediated by different chelators like citrate, nicotinamine and MAs (Kobayashi and Nishizawa 2012). Xylem loading involves efflux transporters and phloem loading was assumed to have influx transporters (Kobayashi and Nishizawa 2012). Efflux and influx transporters play important roles during Fe

translocation. For example, YSL (Yellow Stripe Like) genes are involved in Fe translocation in many plant species (Curie et al. 2001). Plants use different strategies for uptake and storage of Fe and deficiency or toxicity is managed through changes in gene expression for different transporters, Fe storage genes and other associated genes involved in iron metabolism.

Folates

Folate or vitamin B9 provides methyl groups for certain metabolic reactions and deficiency of folate results in anaemia and neural tube defects. Naturally occurring folates are the pteroglutamyl forms of synthetic folic acid. Folic acid is the stable oxidized form (Colman et al. 1975) and natural folates are sensitive to oxidation and are less stable (Murphy et al. 1976). Deficiency of dietary folates may cause neural tube defects in new borns including neural tube defects, spina bifida and anencephaly. Spina bifida is a spinal cord defect and anencephaly is a birth defect where portions of the brain, skull and scalp are partially absent in new borns. Werler et al. (1999) reported that folic acid supplementation of 400 micrograms per day had a positive impact in reducing the number of neural tube defects in newborns. They evaluated 3 basic approaches, folic acid supplements, consumption of folate rich food and fortifying food with synthetic folic acid, to meet the daily intake recommendation by the CDC (US Centers for Disease Control and Prevention). CDC recommendations emphasize daily intake of 400 micrograms of folic acid supplements for all women of child bearing age and to not consume more than 1 mg of folic acid on a daily basis. Women who did not take folic acid supplements usually consumed lower quantities than prescribed. This is due to the fact that bioavailability of food folates was low compared to synthetic folic acid supplements (McNulty and Pentieva 2004). Folate bioavailability differs among foods, for example, egg yolk, liver and orange juice have greater bioavailability than cabbage, lima beans and lettuce (Seyoum and Selhub 1998).

The natural form of folates is the polyglutamyl form and it is hydrolyzed into the monoglutamyl form in the intestine during or before absorption (Halsted and Tamura 1979). Hydrolysis is catalyzed by the conjugase enzyme (EC 3.4.12.10). Two types of folate conjugase are present – an intracellular form and the other is a brush border membrane (brush border membrane is the microvilli covered epithelium cell layer in intestine) bound form (Reisenauer et al. 1977). Folate bioavailability is reduced by factors which impair the conversion of polyglutamyl folates into the monoglutamyl form and prevent absorption through the brush border membrane in the human intestine. For example, trypsin inhibitors, phytohaemagglutinins and different folate binding proteins reduce conjugase enzyme activity (Bhandari and Gregory 1990). The extent of inhibition ranged between null to more than 50% inhibition among different food sources (Bhandari and Gregory 1990; Reisenauer and Halsted 1981).

The most popular method to estimate folate concentration is a microbiological assay involving *Lactobacillus* species. Han and Tyler (2003) used a tri-enzyme extraction and a microbiological assay using *Lactobacillus rhamnosus* L. to estimate total folate concentration in lentil, dry bean and peas. Tri-enzyme (α-amylase, protease, conjugase) extraction is reported to be more efficient than the single enzyme (conjugase) extraction method (Martin et al. 1990). The shortcomings of the microbiological assay to estimate folate concentration resulted in development of a high performance liquid chromatography (HPLC) method that has been adopted by many workers (Gujska and Kuncewicz 2005; Póo-Prieto et al. 2006).

Biofortification

The increase in global food production from the 'Green Revolution' saved millions of lives, however, a steep rise in non-communicable diseases like cardiovascular disease and cancer have been observed (Welch and Graham 1999). This rise in non-communicable diseases is

attributed to nutrient deficiency over a large proportion of the world population. 'Biofortification' has been proposed to combat micronutrient and vitamin deficiencies (White and Broadley 2009, Welch and Graham 1999, 2004). Biofortification involves developing nutrient dense crop plants using plant breeding or modern plant biotechnological tools (White and Boadley 2009) (Fig. 2.1). Originally 'biofortification' was coined by Steve Beebe as a technique to improve the nutritional value of crop plants through genetic selection (Morgan 2013). Johns and Eyzaguirre (2007) reviewed different biofortication programs across the globe on different crop plants, for example canola, cottonseed oil, maize, potato, rice, soybean, sunflower, sweet potato and tomato. They clearly pointed out the necessity of a localized effort in terms of selection of a target crop for nutritional profile improvement as well as the need of increased funding and development of infrastructure to carry out detailed nutritional analysis (Johns and Eyzaguirre 2007). Efforts to improve the nutritional quality should fit well in an ongoing breeding program due to limitation of resources (Johns and Eyzaguirre 2007). One example of a localized and community based biofortification program is the BioCassava Plus Program of CGIAR and other NARS (National Agricultural Research Systems) partners in Africa (Sayre et al. 2011). It was reported that about 250 million people in African nations depend on cassava as the primary daily calorie intake source (Sayre et al. 2011). A typical cassava meal (500 gm) provides sufficient calories, however, it is deficient in micronutrients (example iron, zinc), vitamins (example Provitamin A) and protein (Sayre et al. 2011). The BioCassava Plus program targeted iron, zinc, provitamin A and protein concentration and completion of the first phase resulted in an increase in target nutrients. The ongoing second phase involves confined field trials, release and adoption strategies in African countries like Kenya and Nigeria (Sayre et al. 2011). Meenakshi et al. (2010) critically analyzed the ongoing

HarvestPlus driven biofortification programs across different African and Asian countries with the objective to compare the cost of biofortication programs and impact of realized gains in monetary value. Results showed that biofortication programs are costly and they have a significant positive impact in terms of realized gain (Meenakshi et al. 2010). Nestel et al. (2006) supported the idea of biofortification to alleviate malnutrition along with food fortification or dietary supplementation. Genetic or agronomic biofortification could also be more effective in countries where no coordinated or regulated food fortication program exists.

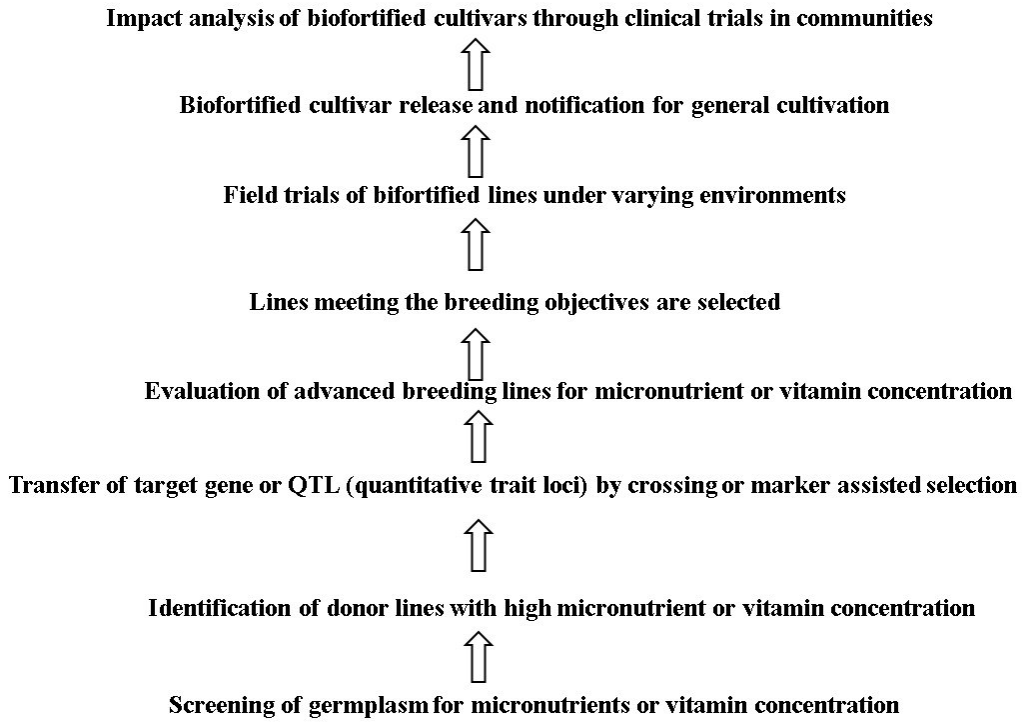

Fig. 2.1. General process of biofortification research.

A recent study in India has shown consumption of iron biofortified pearl millet decreased the iron deficiency in school children (Finkelstein et al. 2015). A Zinc biofortified rice variety (BRRI 72) has been released in Bangladesh for cultivation, this variety is high yielding and has high concentration of zinc (23 mg kg^{-1}). In India, Indira Gandhi Krishi Viswavidyalaya (a land grant agriculture university) released the first high zinc rice variety (Chattisgarh zinc rice 1) for

cultivation. The grain zinc content of this variety is reported to be 6-8 parts per million. Vitamin A biofortified orange maize increased the body storage of vitamin A in Zambian children (Gannon et al. 2014). There are growing evidences that vitamin A biofortified sweet potato is improving the health status of the children in Mozambique (Jones et al. 2015).

Lentil

Lentil (*Lens culinaris* Medik.) is a nutritious food legume crop. Lentil is grown mainly in five different regions of the world; Canada (35%), India, Nepal, and Bangladesh (30%), Turkey and Syria (13%), Australia (8%), and the Midwestern region of the USA (4%) (FAOSTATS 2013). The total world lentil production is about 5 million tons (FAOSTATS 2013) and it is grown over an area of 4.3 million ha. Lentil is one of the earliest plant species domesticated and used in the 'Mediterranean Fertile Crescent' (Cubero 1981). The cultivated lentil was studied in detail by Barulina (1930). Lentil taxonomy is as follows: Kingdom: Plantae (Plants), Subkingdom: Tracheobionta (Vascular Plants), Superdivision: Spermatophyta (Seed plants), Division: Magnoliophyta (Flowering plants), Class: Magnoliopsida (Dicotyledons), Subclass: Rosidae, Order: Fabales, Family: Fabaceae, Genus: *Lens* Mill., Species: *Lens culinaris* Medik. (Ferguson et al. 2000). Lentil is self-pollinated and it has a diploid (2n=14) genome size of about 4 Gb (Arumuganathan and Earle 1991). *Lens* is a small genus of the Viciae tribe and is comprised of only one cultivated species (*L. culinaris* Medik.) and a few wild species or subspecies, including *L. ervoides*, *L. nigricans*, *L. lamottei*, *L. culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus* (Ferguson et al. 2000). *L. orientalis* is the most probable originator of cultivated *L. culinaris* Medik. (Sandhu and Singh 2007). Based on seed size there are two varietal groups of cultivated lentils, *microsperma* and *macrosperma*; *microsperma* is the small-seeded type (seed diameter, 2-6 mm) and *macrosperma* is the large-seeded type (seed diameter,

6-9 mm) (Barulina 1930). *Microsperma* varieties are mostly grown in Asia and Africa and *macrosperma* varieties are grown in the Mediterranean region and North America.

Lentil is considered a cool season crop species and is grown as a summer crop in temperate climates (for example in USA and Canada) and as a winter crop in subtropical climates (for example in India, Pakistan, Nepal). This climatic adaptation is due to the fact that lentil growth is adversely affected above 27°C and it can be grown from sea level up to 3000 m. Lentil is grown under rainfed conditions and requires comparatively colder temperature during initial growth stages (18-25°C) and warmer weather during maturity (25-30°C). Drought and frost tolerance are moderate. The lentil crop is seed propagated and sowing rate and row to row spacing varies from one growing condition to another. It can be grown in loam or clay loam soil and can withstand moderate alkalinity. Lentil is susceptible to any kind of water logging conditions.

Different organizations maintain and preserve the germplasm of cultivated and wild lentil species. The International Centre for Agricultural Research in the Dry Areas (ICARDA, Morocco) developed and maintains a germplasm mini core set comprised of 109 cultivated accessions from 15 countries and 52 wild accessions (*L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *tomentosus* and *L. culinaris* ssp. *odemensis*) from 11 countries (Kumar et al. 2015). Another mini core set of lentil was developed and is maintained by USDA/ARS, Regional Plant Introduction Station, Pullman, USA and is comprised of 384 accessions and is consists of germplasm lines from various countries along with the cultivars, breeding lines and mapping population parents (Simon and Hannan 1995).

Lentil is a potential crop for genetic biofortification due to its rich nutritional profile (Thavarajah and Thavarajah 2012). The narrow genetic base of lentil is similar to other food

legume species, therefore, wild species have to be explored along with cultivated species to find donors for high micronutrient traits. Information regarding mineral trait variability and inheritance is required to initiate an interspecific hybridization program, yet limited nutritional trait information is available for a wide range of *Lens* species.

Sources of high micronutrient and vitamin B9 concentration

Knowledge of available sources of quality traits (micronutrients and vitamins) is prerequisite for a biofortification program and evaluation of genetic resources is necessary to identify suitable sources. A limited number of studies exist in lentil reporting micronutrient and other quality traits. Karaköy et al. (2012) studied the mineral status of Turkish lentil landraces and cultivars in lentil and reported that Fe concentrations ranged between 49.4 to 81.4 mg kg^{-1}. The concentrations reported for Zn, Cu, Ca, and Mg were 46.9-73.1 mg kg^{-1}, 9.1-16.9 mg kg^{-1}, 480-1280 mg kg^{-1} and 850-1260 mg kg^{-1}, respectively (Karaköy et al. 2012). In another study, Solanki et al. (1999) evaluated improved lentil cultivars in India. They reported Fe and Ca concentrations from 80-92 (mg kg^{-1}), and 1150-1650 (mg kg^{-1}), respectively. Thavarajah et al. (2009) reported Fe and Zn concentrations from 73-90 and 44-54 mg kg^{-1}, respectively, in a set of lentil cultivars grown at 9 locations in Canada over 2 years. Zia-Ul-Haq et al. (2011) evaluated four improved lentil cultivars in Pakistan for different micronutrients and reported on Fe, Zn, Cu, and Ca concentrations ranging from 27-32, 39-44, 89-99, and 1180-1210 mg kg^{-1}, respectively. In a study comparing micronutrient concentrations in different legumes, Iqbal et al. (2006) found that Fe, Zn, Cu, Ca and Mg concentration was 31, 44, 99, 1200, and 45 (mg kg^{-1}), respectively, in lentil. Alghamdi et al. (2014) studied 35 advanced breeding lines of cultivated lentil in Saudi Arabia and reported concentrations for Mg (1261-1573 mg kg^{-1}), Ca (64.9-84 mg kg^{-1}), Fe (65.7-85.7 mg kg^{-1}), Zn (26.3 -45.1 mg kg^{-1}), and Cu (8.6 -13.7 mg kg^{-1}).

Recently, Jha et al. (2015) evaluated a set of 4 popular cultivars of each food legume (pea, common bean, lentil and chickpea) from replicated field trials over two locations for folate concentration. The tri –enzyme extraction of seed samples and ultra-performance liquid chromatography coupled with mass spectrometry (UPLC–MS/MS) was used to quantify folate monoglutamate concentrations. Folate concentration ranged between 136.5-182.4 µg/100 g (lentil), 164.6 -232.4 µg/100 g [common bean (*Phaseolus vulgaris*)], 351.5-588.8 µg/100 g [chickpea (*Cicer arietinum*)] and 22.8-29.6 µg/100 g [pea (*Pisum sativum*)]. Significant environment effect on folate concentrations was detected (Jha et al. 2015).

Singh et al. (2015) studied 30 lentil genotypes comprising Mediterranean landraces, breeding lines and released varieties in India for folate concentration. Mean total folate concentration was 222 µg/100 g and ranged from 114.4 to 448.1 µg/100 g. Mediterranean landraces were reported having higher folate concentration compared to other tested lentil genotypes.

Availability of molecular markers in lentil for quality traits

Hundreds of SSRs (simple sequence repeats), EST-SSRs (expressed sequence tagged-single sequence repeats) or SNPs (single nucleotide polymorphisms) (Kaur et al. 2011, 2014; Verma et al. 2013, 2014) have been reported in lentil. Among the validated molecular markers only a limited number of markers were reported to be polymorphic (Hamweigh et al. 2009; Kaur et al. 2011, 2014; Verma et al. 2013, 2014). Hamweigh et al. (2009) developed 14 microsatellite markers from a genomic library developed in lentil genotype ILL5588. Kaur et al (2011) validated a set of 166 EST-SSR markers among which 79 (47.5%) were polymorphic. The test genotypes were 12 cutivated lentils and one wild lentil genotype (*L. nigricans*). In a separate

study, Kaur et al. (2014) reported 61 polymorphic SSRs and 264 SNPs after testing 546 SSRs and 768 SNPs, respectively, in a lentil recombinant inbred line (RIL) population.

Verma et al. (2013) using the transcriptome sequencing and *de novo* assembly analyzed the simple sequence repeats in lentil. Twenty-three primer pairs out of the 54 (42.6%) showed polymorphism while tesing among a set of 24 genotypes comprising lentil, *Glycine*, *Medicago* and *Vigna* genotypes. Amplified alleles ranged between 2-4 and polymorphism information content ranged between 0.06-0.88 with an average of 0.47.

Verma et al. (2014) developed EST-SSRs through transcriptome sequencing of lentil genotype 'Precoz' and validated 33 polymorphic EST-SSRs among 46 lentil and other food legume genotypes. Alleles amplified ranged between 2–5 with an average of 3.73 alleles per locus. Polymorphic information content (PIC) for all the loci ranged from 0.13 to 0.99 with an average of 0.66 per locus.

Recently, Andeden et al. (2015) developed (CA)n, (GA)n, (AAC)n and (ATG)n repeat enriched libraries and by sequencing these libraries found 78 polymorphic SSR markers. Wong et al. (2015) developed genome wide 5389 non-redundant SNPs using a two enzyme genotyping by sequencing (GBS) method.

Ates et al. (2014) mapped 121 QTLs (quantitative trait loci) influencing the uptake of important micronutrients such as Mn and Zn uptake in lentil using a recombinant inbred line (RIL) population derived from the cross between CDC Redberry/ILL7502. In another study (Aldemir et al. 2014), AFLP (amplified fragment length polymorphism), SSR and SNP markers were used for genotyping. Aldemir et al. (2014) reported 4 QTLs controlling iron concentration using 181 molecular markers (150 AFLPs, 27 SSRs and 4 SNPs). The mapping population they

used was a recombinant inbred line population derived from a cross between ILL 8006–BM (Barimasur-4) x CDC Milestone.

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CHAPTER 3. LENTIL-A RICH SOURCE OF FOLATES

Abstract

The potential for genetic biofortification of U.S.-grown lentils (*Lens culinaris* L.) with bioavailable folate has not been widely studied. The objectives of this study were (1) to determine the folate concentration of 10 commercial lentil cultivars grown in Minot and McLean counties, North Dakota, USA, in 2010 and 2011, (2) to determine the genotype (G) × environmental (E) interactions for folate concentration in lentil cultivars, and (3) to compare the folate concentration of other pulses [field peas (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.)] grown in the United States. Folate concentration in lentil cultivars ranged from 216 to 290 μg/100 g with a mean of 255 μg/100 g. In addition, lentil showed higher folate concentration compared to chickpea (42−125 μg/100 g), yellow field pea (41−55 μg/100 g), and green field pea (50−202 μg/100 g). A 100 g serving of lentils could provide a significant amount of the recommended daily allowance of dietary folates (54−73%) for adults. A significant year × location interaction on lentil folate concentration was observed; this indicates that possible location sourcing may be required for future lentil folate research.

Introduction

Folate deficiency is a global problem affecting millions of people in both developed and developing countries (UN, 2008). Inadequate intake of folic acid during pregnancy increases the risks of preterm delivery, low birth weight, fetal growth retardation, and developmental neural tube defects (NTDs). In addition, low folate intake and elevated homocysteine levels are associated with the occurrence of neurodegenerative disorders, cardiovascular diseases, and a range of cancers, while adequate intake of both folates and folic acid in diets decreases total homocysteine levels in plasma (Blancquarert et al. 2010; Ray et al. 2000; Jacques et al. 1999).

Tetrahydrofolate and derivatives, collectively called folates, are water-soluble B-vitamins. Humans and animals cannot synthesize folates, and therefore they must be supplied from plant-based and animal foods including liver and eggs. Pteroylmonoglutamic acid (folic acid) is the synthetic form of folate used in supplements and food fortification. In 1998, US and Canada mandated folic acid enrichment in all grain products to lower the risk of NTDs. This resulted in a 20−53% decrease in the incidence of NTDs and more than a 38% reduction in the prevalence of anencephaly (Blancquarert et al. 2010; Green, 2002). Currently, the recommended daily intake (RDA) of folate is 400 μg of dietary folate equivalent for adults and 600 μg for pregnant women (Institute of Medicine, 1998). Folic acid fortification and supplementation approaches have been adopted in many parts of the world, largely due to folate bioavailability (Blancquarert et al. 2010; Rader 2002). Thus, alternative approaches to supply daily folates through biofortification of staple food crops may provide a sustainable means to provide bioavailable folates to people in many parts of the world (The Office of Dietary Supplements 2012). Most staple food crops, including cereals, potato (*Solanum tuberosum* L.), and banana (*Musa* sp), are poor sources of dietary folates, and diets based on these foods often do not reach the folate RDA of 400 μg/day (UN, 2008; Institute of medicine, 1998). Generally, leafy vegetables contain more folates (1.5−4.5 nmol/g fresh weight) than roots (0.3 nmol/g fresh weight) and fruits (0.2−0.8 nmol/g fresh weight) (Scott et al. 2000). The USDA nutrient database shows lentils (*Lens culinaris* L.) and common beans (*Phaseolus vulgaris* L.) are two pulses that are rich in folates (U. S. Department of Agriculture, 2012). Lentil is a traditional pulse crop mostly grown in low-rainfall, dryland cropping systems in rotation with cereals, wheat and rice. Annual world lentil production is approximately 4.4 M tons, about 90% of which occurs in five specific regions: Canada (35%-1.53 t); India, Nepal, and Bangladesh (30%-1.23 t); Turkey and Syria (13%-0.55 t); Australia

(8%-0.38 t); and the Midwestern region of the USA including North Dakota, South Dakota, and eastern Montana (4%-0.21 t) (FAOSTATS 2010). Lentils are an emerging crop in North Dakota, and Montana, providing economic benefits in addition to the benefits derived from crop rotation, nitrogen fixation, and sustainable agriculture (Northern Pulses Growers Association 2013). Lentil cultivars are grouped into at least six market classes, including extra small red, small red, small green, medium green, large green, and dark green speckled. This classification is based on the size and color of the seed (Thavarajah et al. 2012). Lentils are rich in protein (20− 30%), prebiotics (including fructooligosaccharides, galactooligosaccharide, and resistant starch), and minerals, and are naturally low in phytic acid (Thavarajah et al. 2011; Thavarajah et al. 2009; Johnson et al. 2013a). Therefore, the selection and development of lentils cultivars high in bioavailable folates could have large benefits due to the complementarity profiles of other bioactive molecules present in lentils. To our knowledge, this study is the first comprehensive study on USA-grown lentils to assess their potential as a source of folates for future genetic studies on biofortification. The objectives of this study were (1) to determine the folate concentration of 10 commercial lentil cultivars grown in Minot and McLean counties, North Dakota, USA in 2010 and 2011, (2) to determine the genotype x environment interactions for folate concentration in lentil cultivars and (3) to compare the folate concentration of other pulses [field peas (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.)] grown in the USA.

Materials and methods

Materials

Standards, reagents, and high-purity solvents used for high-performance liquid chromatographic (HPLC) analyses and enzymatic assays were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Folate standards were freshly prepared each day. Water, distilled and

deionized (ddH_2O) to a resistance of ≥18.2 MΩ (Milli-Q Water System, Millipore, Milford, MA, USA), was used for sample extractions and preparation.

Seed samples

The following commercial lentil cultivars were included: CDC Redberry, CDC Red Rider, CDC Lemay, CDC Greenland, CDC Rouleau, CDC Richlea, Riveland, CDC Rosetown, CDC Viceroy, and Pennell (Johnson et al. 2013a). An approximately 250−300 g subsample of seeds was collected. These seed samples were randomly taken from an entire harvested plot with three replications, two locations, and two years (2010 and 2011; total number of samples = 120). Two selected counties were Ward (48° 23′ 25″ N, 101° 29′ 58″ W, 27.2 cm average rainfall, and 17.2 °C mean growing season temperature) and McLean (47° 57′ 74″ N, 101° 239′ 60″ W, 36.3 cm average rainfall, and 17.2 °C mean growing season temperature), North Dakota. Samples were hand-cleaned of debris, air-dried (40 °C), and ground to pass through a sieve size of 0.25 mm using a top-loading UD grinder (Unholtz Dickie Corp., Wallingford, CT, USA). Samples of 10−20 g of ground seed (7.3% moisture) were stored at −40 °C until analysis. The moisture contents of these ground lentil seeds were measured using AACC method 44-15A (AACC International, 2013). In addition, three yellow field pea (DS Admiral, CDC Meadow, and Spider), five green field pea (CDC Striker, Shamrock, SGDP, K2, and Arcadia), and eight chickpea (CDC Frontier, Sierra, Dylan, Dwelley, Bronic, Billy Bean, Troy, and Sawyer) commercial seed samples were collected from the 2012 Pulse Quality Survey (Thavarajah and Thavarajah, 2012). A total of 16 seed samples were collected from North Dakota, Idaho, and Washington. An approximately 500−1000 g subsample of seeds was collected from the 2012 Pulse Quality Survey conducted at the NDSU Pulse Quality and Nutrition Laboratory. Field pea and chickpea samples followed the same processing method as previously described for lentils.

Sample preparation and analysis

Homogenization

A finely ground sample of 0.25 g was weighed and dispersed in 12.5 mL of extraction buffer solution [75 mM potassium phosphate buffer (pH 6.0) containing 52 mM sodium ascorbate and 0.1% (v/v) 2- mercaptoethanol] (Hefni et al. 2010). The mixture was homogenized for 30 s using a vortex mixer. This procedure was done using amber-colored vials under minimum light conditions.

Trienzyme Treatments

The homogenized seed samples were treated with enzymes according to the method described by Hefni et al. (2010). The seed samples were incubated with 1 mL of α-amylase (3000 U/mL) from *Aspergillus oryzae* (EC 3.2.1.1) for 1 h, followed by submersion in a boiling water bath (75 °C) for 12 min, and then cooled on ice. When the samples were cool, 2 mL of protease (5 mg/ mL) from *Streptococcus griseus* (EC 3.4.24.31) was added to each, incubated at 37 °C for 1.5 h, and inactivated by submersion in a boiling water bath for 5 min. Finally, 0.2 mL of conjugase from rat serum was added to each sample, and the samples were incubated at 37 °C for 2.5 h. (Hefni et al. 2010). The enzymes were deactivated by placing the sample in a boiling water bath for 5 min and then cooling it on ice. The samples were centrifuged at 4000 rpm for 15 min, and 1 mL of supernatant was collected in amber-colored bottles (minimum light condition) to reduce the breakdown of isolated folates. Then, these samples were immediately analyzed on a reversed phase high performance liquid chromatograph (RP-HPLC) (Jastrebova et al. 2013). Seed folate concentration was measured by HPLC (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) with a fluorescence detector at excitation and emission wavelengths 290 and 360 nm, respectively. Folates were separated on a C18 column (Prodigy 5 µm, 250 × 4.6 mm

C18 column, Phenomenex, Torrance, CA, USA), with a guard column (Prodigy 5 μm, 30 × 4.6 mm, Phenomenex). The column temperature was maintained at room temperature, 25 ± 1 °C, during the experiment. The mobile phase was acetonitrile and a 30 mM potassium phosphate buffer (pH 2.3) at a flow rate of 0.4 mL/min. The gradient was initiated at 5% acetonitrile and kept for 5 min and then linearly increased to 25% over 20 min. The gradient was kept at 25% acetonitrile for another 6 min. The concentration of the samples was quantified as micrograms of tetrahydrofolic acid (THF) equivalents per 100 g of dry sample. The concentrations of those analyzed THFs were detected within a linear range of 0.1−2.5 μg/g ($r^2 > 0.99$). The minimal detectable limit was 0.01 μg/g. An external laboratory reference, CDC Redberry, was also used daily to ensure the accuracy, sensitivity, and reproducibility of detection. High-resolution mass spectrometry was used to confirm the samples and THF standards using a Bruker Daltonics BioTOF (mode, positive; dry gas temperature, 200 °C; capillary, 4500 V; ionization source, ESI; data reported, m/z) at the NDSU Core Synthesis and Analytical Service Facility, Fargo, ND, USA.

Statistical analysis

The experimental design was a randomized complete block design with three replicates of 10 commercial lentil genotypes grown at two locations over two years (n = 120). For combined analysis, the General Linear Model procedure (PROC GLM) of SAS version 9.3 (SAS Institute Inc., 2008) was used to perform analysis of variance with replicates, locations, and genotypes considered as random factors. A separate analysis of variance was performed for each year using SAS PROC GLM. Means were separated by Fisher's protected least significant difference (LSD) at $P < 0.05$. Lentil folate concentrations were subjected to dissimilarity coefficient analysis using NTSYSpc ver. 2.2. (Rohlf 2009). A dendrogram was constructed

following an unweighted pair group method with arithmetic average (UPGMA) based on a dissimilarity matrix using NTSYSpc ver. 2.2. (Rohlf 2009).

Results

Analysis of variance components

In combined analysis of variance, genotype effects were not statistically significant ($P< 0.05$) (Table 3.1). However, individual location and year specific ANOVA showed that genotypic effects and genotype (G) × environment (E) interactions were significant ($P < 0.05$), with the exception of McClean County in 2011 (Table 3.2). Partitioning of variance further indicated that year × location and year × location × genotype interaction effects were statistically significant ($P< 0.05$) (Table 3.1).

For 2010, total folate concentration ranged from 196 to 329 µg/100 g with an average of 263 µg/100 g over two locations (Table 3.2). For 2011, total folate concentration ranged from 187 to 310 µg/100 g with an average of 249 µg/100 g over two locations (Table 3.2). In this experiment, the total folate concentration in lentils was quantified as tetrahydrofolate (THF).

Total folate density among lentil cultivars

The total folate concentration of lentil cultivars ranged from 216 to 290 µg/100 g with an average of 255 µg/100 g (Table 3.3). A small red cultivar, CDC Rouleau, showed the highest concentration of 290 µg/100 g, and a large green cultivar, CDC Greenland, showed the lowest (216 µg/100 g). Percent recommended dietary intake (%RDA) of folates is 400 µg/day. Therefore, a single serving of 100 g of lentil on a dry weight basis can supply on average 64% of RDA.

Table 3.1. Pooled analysis of variance for folate concentration for 10 lentil varieties grown in North Dakota, USA in 2010 and 2011.

Source	df[a]	Mean square[b]
Genotype	9	9220
Location	1	1904
Year	1	8467
Year*Location	1	399746**
Year*Genotype	9	2545
Location*Genotype	9	13758
Year*Location*Genotype	9	6880**
Error	72	2037

[a] Degrees of freedom based on three replicates. [b] Mean square was significantly different at $P < 0.05$ (**) and $P < 0.1$ (*).

Percent contribution to the folate RDA varies from 54% (CDC Greenland) to 73% (CDC Rouleau) from a single serving of 100 g of lentils (Table 3.3).

Cluster analysis based on folate least-squares means

Ten lentil cultivars were grouped into three clusters based on the mean values generated from unweighted pair group mean average method of analysis (Fig. 3.1). The two cultivars in cluster I, CDC Rouleau and CDC Richlea (287− 290 µg/100 g of folate), had the highest level of folate (Fig. 3.1). Cultivars CDC Rosetown, Pennell, CDC Red Rider, and CDC Viceroy were classified as cluster II with a moderate level of folate (244−269 µg/100 g) (Fig. 3.1). Cluster III consisted of CDC Lemay, Riveland, CDC Redberry, and CDC Greenland with a comparatively lower level of folate (216−228 µg/100 g) (Fig. 3.1).

Table 3.2. Mean concentration of folate and genotype effect by year and location.

Year	Location	folate (µg/100g)[a]	Genotype effect [b]
2010	McLean	196[x]	**
	Ward	329[y]	*
	Mean	263	
	SE	13.94	
2011	McLean	310[x]	NS
	Ward	187[y]	**
	Mean	249	
	SE	13.03	

[a] Means within a column followed by different letters are significantly different at $p < 0.05$(n = 60).
[b] Genotype effect was significantly different at $P < 0.05$ (**) and $P < 0.1$ (*).
NS, not significant.

Table 3.3. Mean folate concentration and % recommended daily allowance (%RDA) of folates from 10 lentil varieties grown in North Dakota, USA, in 2010 and 2011.

Market class	Cultivar	Folate (µg/100g)[a]	% RDA from 100g serving[b]
Small red	CDC Red Rider	252 a	63
	CDC Redberry	219 b	55
	CDC Rouleau	290 a	73
Medium green	CDC Richlea	287 a	72
Extra small red	CDC Rosetown	269 a	67
Large green	Pennell	262 a	66
	CDC Greenland	216 b	54
	Riveland	222 a	56
Small green	CDC Viceroy	244 a	61
Dark green speckled	CDC Lemay	228 b	57
Mean		255	62
SE[c]		13	

[a] Means within a column followed by different letters are significantly different at $P < 0.05$.
b The % RDA for folates (400 µg per day for adults) was calculated based on the 100 g serving of lentils (4). cSE, standard error of combined data (n = 120).

Table 3.4. Comparison of folate concentrations and %RDA from other pulse crops grown in the USA.

Market Class	Cultivar	State	folate (µg/100g)	% RDA from 100g serving
Yellow Peas	DS Admiral	ND	54	14
	CDC Meadows	ND	41	10
	Spider	ND	55	14
	Mean		50	12
	SE		4	
Green Pea	CDC Striker	ND	50	12
	Shamrock	ND	63	16
	SGDP	ND	202	51
	K2	ND	53	13
	Arcadia	ND	156	39
	Mean		105	26
	SE		35	
Kabuli Chickpea	CDC Frontier	ND	125	31
	Sierra	WA	66	17
	Dylan	WA	54	14
	Dwelley	ID	54	14
	Bronic	ID	59	15
	Billy Bean	ID	42	11
	Troy	ID	70	18
	Sawyer	ID	48	12
	Mean		65	16
	SE		8	

Comparison with other food legumes

The total folate concentration in yellow field peas ranged from 41 to 55 µg/100 g with an average of 50 µg/100 g, and green field pea folate concentration ranged from 50 to 202 µg/100 g with an average of 105 µg/100 g (Table 3.4). Chickpea cultivars had folate concentrations ranging from 42 to 125 µg/100 g with an average of 65 µg/100 g (Table 3.4). A 100 g of serving of yellow field peas, green field peas, and chickpeas can supply 12, 26, and 16% of the daily folate intake requirement, respectively (Table 3.4).

Discussion

A folate concentration of 255 µg/100 g (on average) makes lentil a promising whole food source of folates. To our knowledge, this is the first study to quantify total folate levels in lentils in a replicated field study and the first to analyze variance components in a multiyear and multilocation experiment for folate concentration. Nutritional quality traits of most staple food crops including micronutrient and prebiotic concentrations are mostly influenced by genotype (G) × environment (E) interaction (Falcon 2011; Welch and Graham 1999). In 2010, total folate concentration ranged between 196 and 329 µg/100 g over two locations.

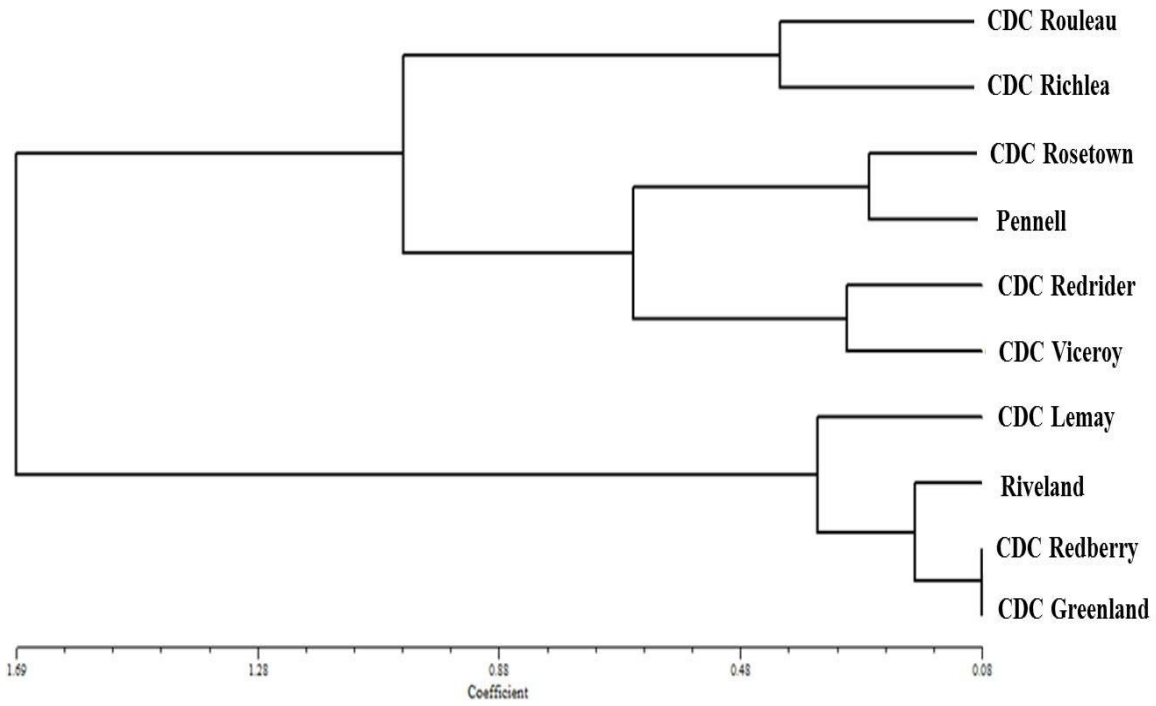

Fig. 3.1. Dendrogram based on dissimilarity matrix data following the unweighted pair group mean average method.

In 2011, total folate concentration varied from 187 to 310 µg/100 g over the locations. In May 2011, the Federal Emergency Management Agency declared both Ward and McLean counties as officially affected by flood damage, and both counties were eligible for public assistance (Federal Emergency Management Agency, 2011). This major meteorological difference between the years contributed to the high year × location and year × location × genotype variance components.

This effect of an interaction component influencing total variances for folate concentration is comparable to the results of several previous studies involving micronutrients, prebiotics, and phenolics in lentil (Johnson et al. 2013a; Johnson et al. 2013b; Thavarajah et al. 2011). The predominance of G × E interaction effects indicates the necessity to include soil fertility analysis of the experimental site particularly before and after the experiment; this analysis will help to evaluate the genetic potential of a genotype for folate concentration more accurately. The grouping of cultivars based on folate concentration will assist in further genetic and agronomic studies for selection and breeding within these lentil market classes. Chickpea and field pea are other cool-season food legumes that are grown extensively in the temperate areas of the world; however, the average concentration of folate (255 µg/100 g) in lentil is higher than in chickpeas and in yellow and green field peas.

This study also indicates that the range of variability within the species is comparatively lower in lentil (216−290 µg/100 g) compared to other food legumes (in the case of kabuli chickpea and field peas folate ranged from 42 to 125 µg/100 g and from 41 to 202 µg/100 g, respectively) (Table 4). The USDA Nutrition Database indicated that total dietary folate equivalences for raw lentils, field peas, and chickpeas are as follows: 479 µg/100 g for lentils, 557 µg/100 g for field peas, and 65 µg/100 g for chickpeas (U.S Department of Agriculture,

2012). Goyer et al. reported that the folate concentration of 12 different common beans grown in different locations of the United States ranged from 202 to 257 μg/100 g, and both of these results are similar to the results reported in this study (Goyar et al. 2008). Food folate levels have been measured using different analytical methods including HPLC and microbial assays (Yarbaeva et al. 2011; Hefni et al. 2010; Goyer et al. 2008; Han et al. 2003). These assays include a microbiological method using *Lactobacillus rhamnosus* (Yarbaeva et al. 2011; Han et al. 2003) and HPLC-MS methods (Yarbaeva et al. 2011; Hefni et al. 2010). Liquid chromatography− mass spectrometry (LC-MS) enables the simultaneous identification and quantification of different folates. In the present study 5-methyl-THF and 10-formyl-THF forms were qualitatively identified to determine the presence of different folate forms. An exhaustive analysis by different excitation/emission by fluorescence detection and use of LC-MS would have provided a range of other folate presence due to the analytical capabilities of those methods. No attempts were made in this study as high-resolution mass spectrometry analysis may not be a feasible high-throughput screening tool due to the time and cost constraints. Therefore, HPLC may be a rapid screening tool when a large number of lentil samples are selected for breeding purposes. Research on folate bioavailability in staple food crops is limited. Food folates are converted to monoglutamyl tetrahydrofolate before absorption in the jejunum. Many factors affect folate bioavailability including folate form, host background, quantity of folate ingested, and nutrient status (Blancquarert et al. 2010). There have been contradictory reports regarding the bioavailability of different folate forms or folic acid. For example, a few studies have suggested that folic acid is more bioavailable than other forms (Gregory et al. 1992). However, other studies reported that there are no significant differences in terms of bioavailability of folic acid and other folate forms (Gregory et al. 1992). Because most of the folate in legumes remains

as THF, the estimates of THF are appropriate as a measure of folate concentrations in lentils (Yarbaeva et al. 2011). Global biofortification efforts for increased levels of micronutrients in lentils have been limited to a few research groups (Johnson et al. 2013a; Thavarajah et al. 2011). A few studies have been reported in which staple crops have been determined to have a useful level of genetic variability for micronutrients including iron, zinc, pro-vitamin A, and carotenoids (Welch and Graham, 2005; Gharam and Welch 2000; Welch and Graham, 1999). On the basis of these initial observations, more detailed future study is suggested to determine the range of genetic diversity that exists in lentil germplasm. This would be helpful to generate data for the entire range of existing genetic variability in this crop species and its close relatives. Furthermore, any future study should also take into consideration the environment and its interaction on genotype effects.

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CHAPTER 4. GENETIC VARIATION OF MINERAL CONCENTRATIONS IN *LENS* GENOTYPES

Abstract

Lentil (*Lens culinaris* Medik.) is an important staple food crop grown in many parts of the world. Information on the seed mineral concentration of genetically diverse *Lens* genotypes is limited. The objective of this study was to determine the genetic variation of iron (Fe), zinc (Zn), calcium (Ca), copper (Cu), and magnesium (Mg) concentrations in 26 lentil accessions representing 4 *Lens* species, and 3 subspecies of *Lens culinaris*. Plants were grown in a greenhouse using a completely randomized design with three replicates. Lentil seed mineral concentration was measured using acid digestion followed by inductively coupled plasma-optical emission spectroscopy. Significant variation in Fe, Zn, Ca, Cu, and Mg concentrations was observed across the different *Lens* species. Seed concentrations of Fe, Zn, Ca, Cu, and Mg varied from 26-92, 17-51, 97-536, 3-12 and 272-892 mg kg^{-1}, respectively. Mineral concentrations for *L. lamottei* (Fe=64-80, Zn=26-40, Ca=311-434, Cu=2-6, Mg=754-839 mg kg^{-1}, respectively), *L. nigricans* (60-70, 33-39, 508-590, 3-4, 445-738 mg kg^{-1}, respectively) and *L. ervoides* (65, 37, 339, 6, 638 mg kg^{-1}, respectively) were within the range of *Lens culinaris* genotypes. No wild species of lentil was found superior to cultivated lentils for all micronutrients studied. The results indicated that the development of intra-specific populations using contrast parents from cultivated species would be better for mapping genes/QTLs associated with mineral nutrient concentrations in lentil.

Introduction

Two billion people around the world suffer from micronutrient malnutrition (IFAD/FAO/WFP 2011). Micronutrient deficiency results from inadequate intake of vitamins and minerals in diets. Different methods are available today to prevent micronutrient malnutrition, including food fortification, dietary supplementation, diversification, and biofortification. Biofortification, using traditional plant breeding practices combined with biotechnology, is a sustainable approach to the development of mineral-dense staple crops (Pfeiffer and McClafferty 2007; Welch and Graham 1999). Biofortification has been a success for several staple food crops including high protein maize (*Zea mays* L.) (QPM), β-carotene rich sweet potato (*Ipomoea batatas*) and rice (*Oryza sativa*), iron (Fe) rich common bean (*Phaseolus vulgaris*) and pearl millet (*Pennisetum glaucum*) cultivars are cultivated in many countries (Bouis et al. 2013). These crop cultivars are gaining popularity among growers in Asia and Africa. White and Broadley (2009) reviewed different mineral biofortification research activities in various crops. They highlighted the potential of agronomic as well as the genetic biofortication to improve the availability of seven mineral traits in human diet, namely, iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg), iodine (I) and selenium (Se) (White and Broadley 2009).

The development of biofortified crop varieties, particularly nutrient rich food legumes, would have a positive impact in alleviating mineral malnutrition in Asian and African nations. Lentil (*Lens culinaris* Medik.) is a popular pulse crop, grown and consumed throughout the world. *Lens* is a small genus belonging to the Fabaceae family of the Viciae tribe. The genus contains one cultivated species (*L. culinaris* subsp. *culinaris*) with three subspecies (*L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus)* and three wild

species (*L. ervoides*, *L. nigricans*, and *L. lamottei)* (Ferguson et al. 2000). Lentil is a potential candidate for mineral biofortification as its nutritional profile is rich in Fe, Zn, and Se (Thavarajah et al. 2011; USDA National Nutrient Database 2015). Identification of mineral dense lentil genotypes is a priority for biofortification research. Karaköy et al. (2012) evaluated mineral concentration of a set of Turkish landraces and cultivated genotypes of lentil and reported considerable genetic variability for Fe, Zn, Cu, Ca, and Mg concentrations. Alghamdi et al. (2014) evaluated 35 advanced ICARDA breeding lines in Saudi Arabia under one field location over two seasons and reported significant variation for Fe, Zn, Cu, Ca, Mg, phosphorus (P), potassium (K), and manganese (Mn) concentrations. However, there is limited information regarding the variation in mineral concentrations among the subspecies of *L. culinaris* and the wild relatives. If high mineral concentrations exist in the subspecies or wild relatives, interspecific hybridization could be used to introgress improved nutritional quality into cultivated lentil (Ladizinsky 1985). The *Lens* subspecies and wild relatives are in use in breeding programs as sources of novel traits such as disease resistance not found in the cultivated lentil (Fiala et al. 2009). Lentil and its wild relatives should be evaluated to determine the variability for mineral concentrations and to identify potential candidate donors. The objectives of this study were to: (a) determine the mineral concentrations of 26 *Lens* genotypes grown under greenhouse conditions, (b) separate lentil genotypes into different groups based on the seed mineral concentration.

Materials and methods

Materials

Chemicals

Chemical reagents and standards used for mineral digestion and analytical determinations were purchased from Alfa Aesar, VWR International and Sigma–Aldrich Co. (St. Louis, MO, USA) and used without further purification. Water (distilled and deionized; ddH_2O) was purified by a Milli-Q Water System (Millipore, Milford, MA, USA) to a resistance of 18.2 MΩ or greater.

Plant materials

The experimental genotypes included 12 *L. culinaris* subsp. *culinaris*, 4 *L. culinaris* subsp. *orientalis*, 3 *L. culinaris* subsp. *tomentosus*, 1 *L. culinaris* subsp. *odemensis*, 1 *L. ervoides*, 3 *L. lamottei* and 2 *L. nigricans* genotypes (Table 4.1). This set of genotypes was selected as it represents different market classes of cultivated lentil as well as the subspecies of *L. culinaris* and the wild relatives. The seeds were obtained from the USDA-ARS Grain Legume Genetics and Physiology Research Unit, WSU, Pullman, Washington, USA and maintained as single plant selections in the former Pulse Quality Laboratory, NDSU, Fargo, ND, USA.

Greenhouse experiment

Ten surface sterilized seeds from each lentil genotype were placed in sterile petri dishes with absorbent filter paper saturated with Millipore filtered water. The petri dishes were placed in the dark at room temperature (22°C). Every second day, the absorbent paper was saturated with 2-3 mL of Millipore water. Plastic pots (15.25cm) were filled with approximately 300 g of a peat- perlite-vermiculite mixture (Sunshine Grow Mix Number 1, Sun Gro Horticulture Canada

Table 4.1. Brief description of 26 genotypes analyzed for five micronutrients.

Species	Genotype/accession	Remark
L. culinaris subsp. *culinaris*	CDC Redberry	small red cultivated type
L. culinaris subsp. *culinaris*	CDC Rosetown	extra small red cultivated type
L. culinaris subsp. *culinaris*	CDC Rouleau	small red cultivated type
L. culinaris subsp. *culinaris*	CDC LeMay	small french green cultivated type
L. culinaris subsp. *culinaris*	CDC Red Rider	medium red cultivated type
L. culinaris subsp. *culinaris*	CDC Greenland	large green cultivated type
L. culinaris subsp. *culinaris*	Barimasur-2	small red cultivated type
L. culinaris subsp. *culinaris*	Barimasur-3	small red cultivated type
L. culinaris subsp. *culinaris*	Barimasur-4	small red cultivated type
L. culinaris subsp. *culinaris*	Riveland	large green cultivated type
L. culinaris subsp. *culinaris*	Eston	small green cultivated type
L. culinaris subsp. *culinaris*	Pennell	large green cultivated type
L. culinaris subsp. *orientalis*	IG72594	small seeded wild type
L. culinaris subsp. *orientalis*	IG72603	small seeded wild type
L. culinaris subsp. *orientalis*	IG72618	small seeded wild type
L. culinaris subsp. orientalis	IG72896	small seeded wild type
L. culinaris subsp. *tomentosus*	IG72830	small seeded wild type
L. culinaris subsp. *tomentosus*	IG72614	small seeded wild type
L. culinaris subsp. *tomentosus*	IG72616	small seeded wild type
L. culinaris subsp. *odemensiss*	IG72688	small seeded wild type
L. ervoides	IG72815	small seeded wild type
L. lamottei	IG110810	small seeded wild type
L. lamottei	IG110812	small seeded wild type
L. lamottei	IG110813	small seeded wild type
L. nigricans	IG72548	small seeded wild type
L. nigricans	IG72549	small seeded wild type

Inc., ON, Canada) and saturated with deionized water. The pots were allowed to drain overnight, and then the weight of each pot recorded. At seeding, three germinated seeds of each lentil genotype were sown in pots at 70% field capacity. A total of 78 pots were seeded: three replicates of the 26 genotypes with randomization among the pots following a complete randomized design. Greenhouse conditions were as follows: day/night temperatures of 22 °C/ 16 °C; photosynthetically active radiation levels of 300 µmol $m^{-2} \cdot s^{-1}$ using a 16 h photoperiod beginning at 0600 local time, and 50-60% relative humidity.Pots were watered to approximately 70% of free draining moisture concentration every day and 250 mL of nutrient solution were

added to all pots every two weeks. Nutrient concentrations of the all-purpose Plants-Prod 20-20-20 Classic fertilizer solution (Plant Products Co. Ltd., Brampton, ON, Canada) were 20% total N, 20% total P, 20% soluble K, 0.02% B, 0.05% chelated Cu, 0.1% chelated Fe, 0.05% Mo, 0.05% Zn, and 1% EDTA. Plants were thinned to two per pot after one week. Plants were harvested at physiological maturity and threshed individually. Seeds were ground using a stainless steel coffee grinder to obtain fine quality flour.

Mineral concentration

Mineral (Fe, Zn, Cu, Ca, Mg) concentrations in lentil seeds were determined using a previously described modified HNO_3-H_2O_2 method (Alcok et al. 1987; Thavarajah et al. 2009). Finely ground seed samples (500 mg) were placed in individual digestion tubes. Six mL of concentrated (70%) nitric acid (HNO_3) was added to each digestion tube. The digestion tubes were placed in a 90 °C digestion block for one hour and they were shaken at 15 and 45 minutes. Three mL of 30% w/w hydrogen peroxide (H_2O_2) was then added to each tube. The tubes were kept for 15 m at 90 °C temperature. Finally, 3 mL of 6 M hydrochloric acid (HCl) was added to each digestion tube, and the tubes were kept in the digestion block for 5 minutes. Upon complete digestion (the time required for complete digestion was determined in earlier laboratory experiments, the complete digestion is indicated by the discontinuation of brown smoke coming out from the digestion tube), the tubes were removed from the digestion block, the volume was adjusted to 10 mL, and then filtered (Whatman No. 1 filter papers) using a vacuum system (Gardener Denver Thomas Inc., Welch Vacuum Technologies, LA, USA). Mineral concentrations of the filtrates were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES); ICP-6500 Duo, Thermo Fisher Scientific, Pittsburg, PA, USA). Plasma settings of the ICP were, flush pump rate-75 rpm, analysis pump rate- 50 rpm, pump stabilization

time- 5 s, pump tubing type- orange/white tygon. Source setting of the ICP were, RF powar-1150, auxillary gas flow- 1 litre/min, nebulizer gas flow- 0.7 litre/min. Sample flush time of the ICP was 10 s with 3 repeats and plasma view was in autoview mode. Measurements of total minerals were validated using National Institute of Standards and Technology (NIST) standard reference material (SRM) 1576a (wheat flour; [Fe]=14.11±0.13 mg kg^{-1}, [Zn]=11.61±0.26 mg kg^{-1}, [Ca]=191.4±3.3 mg kg^{-1}, [Mg]=398±12 mg kg^{-1}, [Cu]=2.03±0.14 mg kg^{-1}). Calibration curves for Fe, Zn, and Cu concentration were made using serial dilutions from 0.5 to 50.0 mg L^{-1}. The detection limit was 5 µg L^{-1}. Calibration curves for Ca and Mg concentration were made using serial dilutions from 10 to 500 mg L^{-1}.

Statistical analysis

The experimental design was a completely randomized design (CRD) with three replicates of 26 *Lens* genotypes (n=78). Analysis of variance was performed using the General Linear Model (PROC GLM) of SAS version 9.3 (SAS Institute, 2009). Means were separated using Fisher's protected least significant difference (LSD) at $P < 0.05$. Lentil mineral concentrations were subjected to dissimilarity coefficient analysis using NTSYSpc ver. 2.2 (Rohlf 2009). Cluster analysis following an unweighted pair group method with arithmetic average (UPGMA) based on a dissimilarity matrix data was performed using NTSYSpc ver. 2.2. A dendrogram was developed using cluster analysis.

Results

Mean Fe concentration was 61 mg kg^{-1} across all 26 lentil genotypes tested (Table 4.2). Among the 20 *L. culinaris* genotypes, Fe concentration ranged from 26 (IG72830) to 92 mg kg^{-1} (CDC Red Rider) with a mean of 58 mg kg^{-1}. CDC Redberry and CDC Red Rider had a significantly higher concentration of Fe compared to other tested genotypes. Fe concentration

was significantly lower in the genotypes belonging to different *L. culinaris* subspecies (*L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus*) than in improved cultivars or breeding lines (*L. culinaris* subsp. *culinaris*). *L. lamottei* genotype IG110810 had a significantly higher concentration (80 mg kg^{-1}) of Fe compared to other non-*culinaris* wild types. All the non-*culinaris* wild type genotypes differed significantly in terms of Fe concentration except IG110812 (*L. lamottei*) and IG72815 (*L. ervoides*). Notably, CDC bred cultivars had significantly higher Fe concentrations than the Barimasur series, with the exception of Eston. Percent recommended daily allowance (RDA) of Fe for the genotypes evaluated ranged from 14-51% per serving.

For the 26 lentil genotypes evaluated, the mean Zn concentration was 33 mg kg^{-1} (Table 4.2). Zn concentration ranged from 17 (IG72830) to 51 mg kg^{-1} (CDC Rosetown) among the 20 *L. culinaris* genotypes with a mean of 32 mg kg^{-1} (Table 4.2). Within *L. culinaris* subsp. *culinaris* genotypes CDC Rosetown (51 mg kg^{-1}) had a significantly higher concentration of Zn compared to other genotypes. Among the other subspecies (*L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus*) IG72614 (43 mg kg^{-1}) had a significantly higher concentration of Zn compared to other genotypes. All the non-*culinaris* wild type genotypes differed significantly in terms of Zn concentration. Means within a column followed by different letters are significantly different at $P< 0.05$ (n = 78), Percent RDA values were calculated with daily requirement of 18 mg of Fe and 8 mg of Zn (females, age 19+ years) (Otten et al. 2006). Percent RDAs were calculated based on the serving size of 100 g of dry

Table 4.2. Mean iron (Fe) and zinc (Zn) concentrations for 26 genotypes and the fraction of the recommended daily allowance (RDA) that each genotype would supply based on 100 g serving size.

Genotype	Fe concentration (mg·kg-1)	%RDA	Zn concentration (mg·kg-1)	%RDA
CDC Redberry	91 a	51	37 c,d	46
CDC Rosetown	82 a,b,c	46	51 a	64
CDC Rouleau	71 a,b,c,d,e	39	46 a,b	58
CDC LeMay	68 b,c,d,e,f	38	31 d,e,f,g,h	39
CDC Red Rider	92 a	51	45 a,b	56
CDC Greenland	64 c,d,e, f,g,	36	43 b,c	54
Barimasur-2	52 e,f,g,h,i,j	29	33 d,e,f,g	41
Barimasur-3	46 f,g,i,j,k,h	26	31 d,e,f,g,h	39
Barimasur-4	36 i,j,k	20	25 h,i,j	31
Riveland	62 c,d,e,f,g,h	34	30 e,f,g,h,i	38
Eston	39 h,i,j,k	22	17 k	21
Pennell	87 a,b	48	36 c,d,e	45
IG72594	54 e,f,g,h,i	30	33 d,e,f	41
IG72603	34 j,k	19	18 k	23
IG72830	26 k	14	17 k	21
IG72688	36 i,j,k	20	22 j,k	28
IG72614	58 d,e,f,g,h	32	43 b,c	54
IG72616	61 c,d,e,f,g,h	34	36 c,d,e	45
IG72618	43 g,h,i,j,k	24	24 i.j.k	30
IG72896	67 b,c,d,e,f	37	26 g,h,i,j	33
IG110810	80 a,b,c,d	44	26 f,g,h,i,j	33
IG110812	64 b,c,d,e,f,g	36	40 b,c	50
IG110813	70 a,b,c,d,e	39	31 b,c	39
IG72548	60 c,d,e,f,g	33	28 f,g,h,i,j	35
IG72549	71 a,b,c,d,e	39	33 d,e,f	41
IG72815	65 b,c,d,e,f,g	36	37 c,d,e	46
Mean	61	39	33	41
SE	2.4		1.1	
Range	26-92	14-51	17-51	21-64

lentil. Among the non-culinaris wild types, IG110812 (40 mg kg-1) had significantly higher concentration of Zn. There was no significant difference for zinc concentration between CDC

cultivars and Barimasur series. Each serving of lentil accounts for 21-64% of RDA of Zn (8 mg) (Otten et al. 2006).

The mean Cu concentration across all lentil genotypes was 6 mg kg^{-1} (Table 4.3). Cu concentration among the 20 *L. culinaris* genotypes ranged from 2.6 (IG72688) to 12.0 mg kg^{-1} (CDC Rosetown) with a mean of 6 mg kg^{-1}. Within *L. culinaris* subsp. *culinaris* genotypes, CDC Rosetown (12 mg kg^{-1}) had significantly higher concentration of Cu compared to other genotypes. Among the other subspecies (*L. culinaris* subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus*), genotypes belonging to *tomentosus* subspecies, IG72614 (12 mg kg^{-1}) and IG72616 (12 mg kg^{-1}) had significantly higher concentration of Cu than other genotypes. Among the non-*culinaris* wild type genotypes IG110812 (6 mg kg^{-1}) and IG72815 (6 mg kg^{-1}) recorded significantly higher concentration of Cu. CDC cultivars had significantly higher Cu concentrations than the Barimasur series (except Eston, 4 mg kg^{-1}). Tested lentil genotypes have the potential to meet 22-133% of the Cu RDA (0.9 mg) (Otten et al., 2006) per serving.

Among all evaluated lentil genotypes, the mean Ca concentration was 339 mg kg^{-1} (Table 4.3). Mean Ca concentration among the 20 *L. culinaris* genotypes was 323 mg kg^{-1}, with the lowest concentration in Eston (97 mg kg^{-1}) and the highest in Pennell (536 mg kg^{-1}). Within *L. culinaris* subsp. *culinaris* genotypes, Pennell (536 mg kg^{-1}) had significantly higher concentration of Ca compared to other genotypes. Genotype from the *orientalis* subspecies [IG72594 (534 mg kg-1)] had a significantly higher concentration of Ca than other genotypes. Among the non-culinaris wild type genotypes belonging to *L. nigricans*, IG72548 (508 mg kg-1) and IG72549 (590 mg kg-1), had a significantly higher concentration of Ca. There was no

Table 4.3. Mean copper (Cu), calcium (Ca) and magnesium (Mg) concentration of 26 genotypes and for 26 genotypes and the fraction of the recommended daily allowance (RDA) that each genotype would supply based on 100 g serving size.

Genotype	Cu ($mg \cdot kg^{-1}$)	%RDA	Ca ($mg \cdot kg^{-1}$)	%AI	Mg ($mg \cdot kg^{-1}$)	%RDA
CDC Redberry	10 b	111	323 b,c,d,e	3	272 l	9
CDC Rosetown	12.0 a	133	257 e,f	3	423 j,k,l	14
CDC Rouleau	9.0 b,c,d	100	318 b,c,d,e	3	556 h,i,j	18
CDC LeMay	7 d,e,f	78	409 b	4	842 a,b,c	27
CDC Red Rider	10 b	111	361 b,c	4	656 d,e,f,g,h,i	21
CDC Greenland	9.0 b,c,d	100	205 f,g	2	610 f,g,h,i	20
Barimasur-2	4.0 g,h,i,j	44	337 b,c,d,e	3	697 c,d,e,f,g,h,i	22
Barimasur-3	4.0i,j,k	44	314 b,c,d,e	3	707 b,c,d,e,f,g,h	23
Barimasur-4	6.0 f,g,h,i	67	344 b,c,d,e	3	662 d,e,f,g,h,i	21
Riveland	8.0 b,c,d	89	355 b,c,d	4	537 1i,j	17
Eston	4.0 g,h,i,j	44	97 h	1	331 1k,l	11
Pennell	7.0 d,e,f	78	536 a	5	892 a	29
IG72594	3.0 j,k	33	534 a	5	584 g,h,i,j	19
IG72603	3.0 j,k	33	313 b,c,d,e	3	581 g,h,i,j	19
IG72830	4.0 j,k	44	112 g,h	1	375 1k,l	12
IG72688	3.0 j,k	33	352 b,c,d,e	4	643 e,f,g,h,i	21
IG72614	6.0 e,f	67	304 c,d,e	3	807 a,b,c,d	26
IG72616	6.0 e,f,g	67	341 b,c,d,e	3	865 a,b	28
IG72618	4.0 h,i,j	44	264 d,e,f	3	540 i,j	17
IG72896	3.0 j,k	33	368 b,c	4	732 a,b,c,d,e,f,g	24
IG110810	2.0 k	22	357 b,c,d	4	754 a,b,c,d,e,f	24
IG110812	6.0e,f,g,h	67	311 c,d,e	3	789 a,b,c,d,e	25
IG110813	5.0 e,f	56	434 c,d,e	4	839 a,b,c,d	27
IG72548	4.0 i,j,k	44	508 a	5	738 a,b,c,d,e,f,g	24
IG72549	3.0 j,k	33	590 a	6	445 j,k	14
IG72815	6.0 e,f	67	292 c,d,e,f	3	756 a,b,c,d,e,f	24
Mean	6	67	339	3	638	21
SE	0.3		14		21	
Range	2-12	22-133	97-590	1-6	272-892	9-29

Means within a column followed by different letters are significantly different at $P< 0.05$ (n = 78), Percent RDA were calculated with daily requirement of 900 µg for Cu, 1000 mg for Ca, and 310 mg for Mg (females, age 19+) (Otten et al. 2006). Percent RDAs were calculated based on the serving size of 100 g of dry lentil. For Ca, Adequate Intake (AI) values are available, not the RDA (Otten et al. 2006).

significant difference for Ca concentration between the CDC cultivars and the Barimasur series. Percent RDA (1000 mg) of Ca (Otten et al 2006) ranged from 1-6% per serving.

The mean Mg concentration among all tested lentil genotypes was 638 mg kg^{-1} (Table 4.3). Magnesium concentration ranged between 272 (CDC Redberry) and 892 mg kg^{-1} (Pennell) among the 20 *L. culinaris* genotypes, with a mean of 616 mg kg^{-1}. Pennell had the highest Mg concentration of all *L. culinaris* subsp. *culinaris* genotypes tested. Within *L. culinaris* subsp. *culinaris* genotypes, Pennell (892 mg kg^{-1}) had a significantly higher concentration of Mg compared to other genotypes. Genotypes from the *tomentosus* subspecies [IG72614 (807 mg kg^{-1}) and IG72616 (865 mg kg^{-1})] had a significantly higher concentration of Mg than other genotypes. Genotypes belonging to *L. lamottei*, IG110813 (839 mg kg^{-1}) had a significantly higher concentration of Mg compared to other genotypes. The Barimasur series did not significantly differ in Mg concentration from the CDC cultivars. Percent RDA (310 mg) of Mg (Otten et al 2006) ranged from 9-29% per serving.

Cluster analysis based on the mineral concentrations

Based on the cluster analysis, five groups were formed (Fig. 4.1). Cluster I consisted of only one genotype, CDC Redberry, which had a unique mineral profile. Cluster II contained two genotypes, Eston and IG72830. The genotypes constituting Cluster III were: CDC Rosetown, CDC Rouleau, CDC Greenland, Riveland, IG72603, and IG72618. The largest cluster, Cluster IV, consisted of CDC LeMay, CDC Red Rider, Pennell, IG72548, Barimasur-2, Barimasur-3, Barimasur-4, IG72614, IG72616, IG72688, IG110810, IG110812, IG110813, IG72815, IG72896. Cluster V contained two genotypes, IG72594 and IG72549.

Discussion

Lentil is a cool season food legume with a narrow genetic base, therefore genetic variability for individual traits is generally low (Eujayl et al. 1998). This was shown for recently improved lentil cultivars with similar or identical pedigrees (Kumar et al. 2004). Interspecific hybridization, either directly between cross compatible species or indirectly between cross-incompatible species using a bridge species, can be used in the genetic improvement of lentils (Kumar et al. 2011). This technique is utilized when a desirable characteristic is present in another related or crossable species (Tullu et al. 2011).

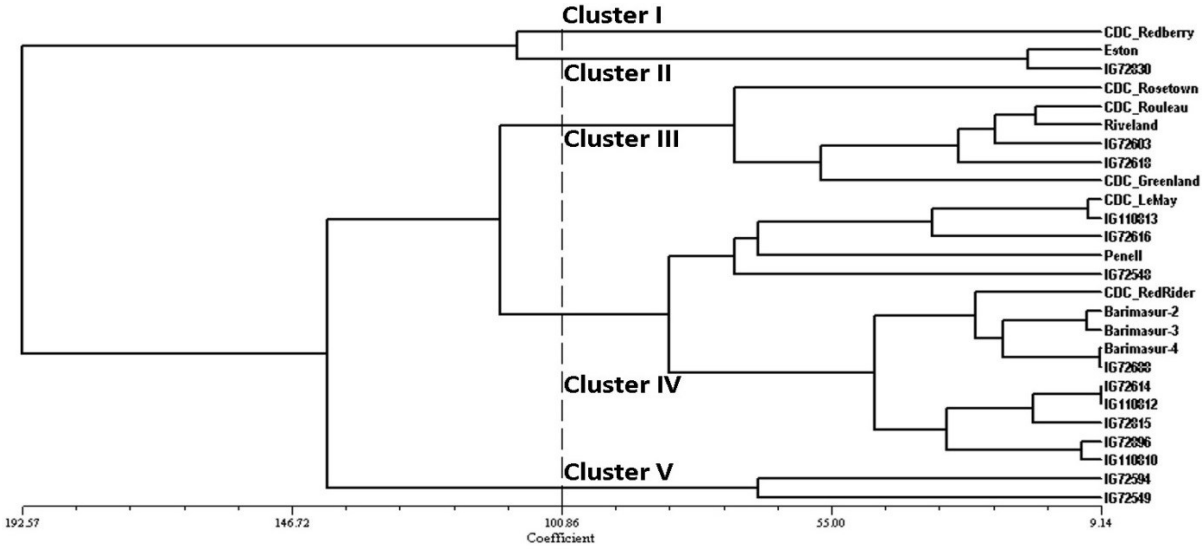

Fig. 4.1. Dendrogram based on mineral concentrations of 26 *Lens* genotypes following unweighted pair group mean average method. Five clusters were formed based on mineral concentrations (Fe, Zn, Cu, Ca and Mg) of the 26 genotypes.

Biofortification for mineral traits is a priority research area in food legumes (including lentil) (Grusak 2009; Thavarajah et al. 2009, 2011; Johnson et al. 2013; Iqbal et al. 2006; Hunt 2003). Development of genotypes with higher concentrations of mineral nutrients is important to allow lower quantities of lentil would to be consumed to meet the recommended daily allowance (USDA National Nutrient Database 2015). Thus, lower intake requirements are important from a

practical as well as economic point of view. Wild species and subspecies of a genus are usually a poor source for mineral traits, however, crossing two different species or subspecies may generate transgressive segregants due to accumulation of additive genes. The selection of genotypes based on concentration of micronutrients (Fe, Zn, Cu, Ca, Mg) (Tables 4.2 and 4.3) could be utilized to develop intraspecific or interspecific mapping populations. While making interspecific crosses the cross-compatibility has to be taken into consideration. The primary gene pool members are easily cross-compatible (*Lens culinaris* subsp. *culinaris*, *Lens culinaris* subsp. *odemensis*, *Lens culinaris* subsp. *orientalis*, *Lens culinaris* subsp. t*omentosus*) (Ferguson et al. 2000). Crossing between primary and secondary/tertiary gene pools members (*L. ervoides*, *L. lamottei*, *L. nigricans*) may not be successful or require use of tissue culture based techniques like embryo rescue or use of bridge species in making crosses (Ferguson et al. 2000).

In the present study, significant variation in mineral (Fe, Zn, Cu, Ca, Mg) concentration was observed. Similarly, Karaköy et al. (2012) studied the mineral status of Turkish lentil landraces and cultivars in lentil and reported Fe concentration from 49.4 to 81.4 mg kg^{-1}. The concentrations reported for Zn, Cu, Ca, and Mg were 46.9-73.1 mg kg^{-1}, 9.1-16.9 mg kg^{-1}, 480-1280 mg kg^{-1} and 850-1260 mg kg^{-1}, respectively. Similarly, Solanki et al. (1999) evaluated improved lentil cultivars in India and reported Fe and Ca concentration from 80 to 92 (mg kg^{-1}), and 1150 to 1650 (mg kg^{-1}), respectively. The Ca concentrations Solanki et al. (1999) reported were higher than those from the present study, possibly due to the genotypic differences in Indian lentil cultivars and or different soil conditions. Thavarajah et al. (2009) reported Fe and Zn concentrations in the range of 73-90 and 44-54 mg kg^{-1}, respectively, in a set of lentil cultivars grown in 9 locations in Saskatchewan, Canada over 2 years. The present study demonstrated more variation for these two micronutrients, which is attributed to the inclusion of

related species in addition to *L. culinaris*. Zia-Ul-Haq et al. (2011) evaluated four improved lentil cultivars in Pakistan for different micronutrients and reported that Fe, Zn, Cu, and Ca concentration ranged from 27-32, 39-44, 89-99, and 1180-1210 (mg kg^{-1}), respectively. In a study comparing micronutrient concentrations in different legumes, Iqbal et al. (2006) found that Fe, Zn, Cu, Ca and Mg concentration was 31, 44, 99, 1200, and 45 (mg kg^{-1}), respectively, in lentil. In these studies, the reported Fe concentration was low and the Ca and Cu concentrations were high compared to the concentrations observed in the present study. The differences may be due to the fact that seeds were not from the single uniform trials, as no information is available from these reports with regard to how plants were grown in the field or greenhouse. In addition, differences might be due to their use of less sensitive or accurate flame/graphite atomic absorption spectrophotometer (AAS) instrument, compared to the more sensitive ICP-OES, to determine micronutrients. AAS is more vulnerable to physical and chemical interferences compared to ICP-OES. Alghamdi et al. (2014) studied 35 advanced breeding lines of cultivated lentil in Saudi Arabia from a field trial over two years and reported concentrations for Mg (1261-1573 mg kg^{-1}), Ca (64.9-84 mg kg^{-1}), Fe (65.7- 85.7 mg kg^{-1}), Zn (26.3 -45.1 mg kg^{-1}), and Cu (8.6 -13.7 mg kg^{-1}). This corresponds closely to the concentrations of Fe, Zn, and Cu but not for Mg and Ca concentration reported in the present study.

Cluster analysis grouped the genotypes into five groups. CDC Redberry, the sole member of Cluster I, has a unique mineral profile with high Fe, low Zn, high Ca, moderately high Cu and low Mg concentrations compared to the other genotypes. For the other clusters the grouping of genotypes based on their mineral concentrations parallels their taxonomic designations (Table 4.1).

The Food and Nutrition Board of the Institute of Medicine, USA established percent recommended daily allowance (RDA) for the minerals (Otten et al., 2006). The RDA is the average recommended daily level of intake of a particular nutrient that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy people (Otten et al. 2006). The values vary by age and gender and in this study, the RDA used for calculations are for females, 19 to 50 years old. This class of individuals was chosen because for most of the minerals daily intake requirements were higher compared to other age groups. Percent RDA values were calculated based on a 100 g serving size of dry lentils for each of the minerals (Otten et al. 2006). A considerable proportion (for Fe 14-51%, for Zn 21-64%, for Cu 22-133%, for Mg 9-29%) of RDA for minerals would be obtained from consuming 100 g of dry lentils (Table 4.2 and 4.3) which is similar to data reported in previous studies (Thavarajah et al. 2009, 2011). Percent RDA of Ca was only 1-6% in the case of tested lentil genotypes which indicates lentil as not a good source to meet the daily requirements of Ca. Developing lentil varieties with high concentrations of Fe and Zn would be especially beneficial for those parts of the world (Asian and African countries) where 40-45% of school-age children are Fe- and Zn-deficient (de Benoist et al. 2008).

Lentils are an integral part of diets in many countries in Asia, including Bangladesh, Nepal, India, and Pakistan. People living in these areas are affected with mineral deficiencies, particularly iron deficiency. Biofortification of minerals in lentil will have a positive impact on maternal and child health in these mineral deficient areas. Cluster analysis based on the overall mineral profile grouped the tested genotypes into five clusters that corresponded to their genetic relatedness. None of the groups had high concentrations of all the minerals (Fe, Zn, Cu, Ca and Mg). This information could be of potential use for intra- or interspecific hybridization in lentil

for quality traits. While this study is not exhaustive, it may serve as a caution for potential linkage drag on seed mineral nutrient concentration when introgressing a desired trait, e.g. disease resistance from the *Lens* subspecies or wild relatives into current cultivars. Utilization of different genotypes with very high and very low mineral concentrations identified may result in the generation of mapping populations that could be used for mapping gene(s) or QTL(s) controlling these micronutrients in lentil. Genomic approaches (Kaur et al. 2011; Verma et al. 2013; Sharpe et al. 2013; Wong et al. 2015) could be used to map or tag genes involved for these mineral traits in lentil and for precision introgression of novel traits from the *Lens* species and wild relatives.

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CHAPTER 5. GENETIC DIVERSITY ANALYSIS OF CULTIVATED AND WILD LENTIL SPECIES

Abstract

Lentil (*Lens culinaris* Medikus) is an important food legume grown extensively throughout the world. This study investigated the genetic relatedness of 29 *Lens* genotypes using simple sequence repeat (SSR) marker-based genotyping. Tissue samples were collected from two-week old seedlings. Twenty-nine *Lens* genotypes were fingerprinted with 39 SSR markers. Thirteen markers were polymorphic among the test genotypes. Thirteen SSRs grouped the 29 *Lens* genotypes, based on their genetic relatedness, into four clusters. Jaccard similarity coefficients ranged between 0.31-0.72. Polymorphic information contents ranged from 0.18-0.64 and average number of alleles amplified per marker was three. Percent variability explained by individual principal components indicated significant diversity. This study demonstrated genetic relatedness among different species of *Lens*.

Introduction

Lentil (*Lens culinaris* Medik.) is a popular food legume consumed heavily in India, Bangladesh, Nepal and many other parts of the world. With the recent trend of gluten-free food products and healthy diet charts, lentil is being introduced extensively to restaurant menus in many parts of the world. Lentil is a high protein, mineral, vitamin, and energy crop with many nutritional benefits (Thavarajah et al. 2011; Sen Gupta et al. 2013). Lentil is mainly grown in Canada, India, Australia, Turkey, USA, Bangladesh, Syria, Iran, Ethiopia and Nepal. Initially, only small to medium sized 'Persian' types were introduced for cultivation in the USA where the primary production areas are Montana, North Dakota, Idaho and Washington. In 2014-15, US grown lentils were expoted to India (33%), Spain (14%), Peru (5%), Mexico (5%), Canada (4%),

other latin-American (<10%) and South-Asian countries (<10%) and the Middle East (<10%) (USDA Economic Research Service Database 2015). USA produced 0.23 MT with average production of 1621 kg/ha (FAOSTAT 2013). The world production of lentil was 4.95 MT with average yields of 1139 kg/ha (FAOSTAT 2013).

Lentil (*Lens culinaris* Medik.) is a member of the leguminosae family, and was derived from the wild progenitor species *Lens culinaris* subsp. *orientalis* (Ferguson et al. 2000). *Lens* is comprised of only one cultivated species (Medik.) and several wild species or subspecies, including *L. ervoides*, *L. nigricans*, *L. lamottei*, *L. culinaris* subsp. *orientalis*, and *L. culinaris* subsp. *tomentosus* (Ferguson et al. 2000).

Molecular markers are useful to assess genetic diversity in crop species including the food legumes (Udupa et al. 1999; Reddy et al. 2010). Transcriptome sequencing or marker transferability have generated hundreds of markers in lentil, however, availability of polymorphic SSR markers and their use in the assessment of genetic diversity is still limited in lentil compared to other food legumes such as chickpea and pigeonpea (Hamwieh et al. 2005, 2009; Kaur et al. 2011, 2014; Datta et al. 2011; Verma et al. 2014). Hamweigh et al. (2009) developed 14 microsatellite markers from a genomic library developed in lentil genotype ILL5588. Kaur et al (2011) validated a set of 166 EST-SSR markers among which 79 were polymorphic. Kaur et al. (2014) reported polymorphic 61 SSRs and 264 SNPs after testing 546 SSRs and 768 SNPs in lentil.

In another study, twenty-three primer pairs showed polymorphism in a set of 24 genotypes comprising lentil, *Glycine*, *Medicago* and *Vigna* genotypes (Verma et al. 2013). Verma et al. (2014) developed EST-SSRs by transcriptome sequencing and validated 33 polymorphic EST-SSRs among 46 lentil and other food legume genotypes. Recently, Andeden et

al. (2015) developed 78 polymorphic SSR markers in lentil. The objective of the current experiment was to assess population structure of 29 genotypes across multiple *Lens* species using SSR markers.

Materials and methods

Plant materials

Twenty-nine *Lens* genotypes were used [CDC Maxim, CDC Rouleau, Barimasur-4 (Sarker et al. 1999), CDC LeMay, CDC Viceroy, Eston, WA8649090 (Kahraman et al 2004a), CDC Rosetown, PI 572359, CDC Richlea, CDC Redberry(Vandenberg et al. 2006), PI 320937, Precoz (Kahraman et al 2004b), CDC Greenland, Pennell (Muehlbauer and McPhee 2004), Riveland (McPhee and Muehlbauer 2009), CDC Red Rider, IG 72618, IG 72688, IG 72549, IG 72603, IG 72830, IG 72594, IG 110813, IG 72614, IG 110812, IG 110810, IG 72616, and IG 72896] (Table 5.1). The seed materials were obtained from the former Pulse Quality and Nutrition Laboratory of North Dakota State University (NDSU), Fargo, North Dakota, USA and the Grain Legume Genetics and Physiology Research Unit, USDA-ARS, WSU, Pullman, Washington, USA.

Genotyping of plant materials

Plant tissue samples were collected from two week old seedlings of individual genotypes. DNA extraction was carried out using a DNeasy® Plant Mini Kit (Qiagen, Valencia, CA, USA), and DNA concentrations were quantified using a Nanodrop 2000c spectrophotometer (Nanodrop, Wilmington, DE, USA). The extracted DNA samples were diluted to a uniform concentration of 20 µg/µL for subsequent polymerase chain reaction (PCR) amplification. Thirty-nine SSR primer pairs (Table A1) developed by genome or transcriptome sequencing of *Medicago* (*Medicago truncatula* Gaertn.) and/or lentil (Gupta et al. 2012; Kaur et al. 2011) were

synthesized by Integrated DNA Technologies (Coralville, IA, USA). The PCR reactions (25 µL volume) were conducted in an ABI 7500 (Applied Biosystems, Foster, CA, USA) thermocycler. Each reaction contained 2.5 µL Taq buffer (Sigma, USA), 1.5 µL $MgCl_2$ (25 mM) (Sigma, USA), 0.20 mM of each dNTP (Sigma, USA), 0.50 mM of each primer (IDT, USA), 0.25 µL Hot Start Taq polymerase (Sigma, USA), and 20 ng of template DNA. Touchdown PCR conditions were 95 °C for 10 min, followed by 10 cycles of 94 °C for 30 s, 60-50 °C for 30 s, 72 °C for 30 s followed by 25 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s and a final elongation step of 72 °C for 10 min (Kaur et al. 2011). The PCR products were resolved in 3% agarose gel (molecular biology grade; Sigma, USA) with bands scored using a gel documentation system. Polymorphism information content (PIC) values of the informative markers were calculated using PICcalc (Nagy et al. 2012).

Statistical analysis

Bands were scored for presence or absence as '1' and '0', respectively. The binary data matrix was subjected to statistical analysis using NTSYS version 2.21q software (Rolhf 2009) following the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean). Principal component analysis (PCA) using NTSYS ver. 2.21q was performed to determine the percent variation explained by individual components.

Table 5.1. Description of *Lens* genotypes used for genotyping with 39 SSR markers.

Genotype	Species	Pedigree description	General description	Reference
CDC Redberry	*Lens culinaris* Medik. subsp. *culinaris* .	Cross between 1049F_3 / 819-5R. Line 1049F_3 was derived from the cross 567-16/545-8. Line 819-5R was derived from the cross 86-360/(458-258G(458-122/C8L27-RC//Precoz)F_2)F_1.	Improved cultivar maintained by Crop Development Centre, University of Saskatchewan, Saskatoon, Canada	Vandenberg et al. 2006
CDC Rosetown	*L. culinaris* Medik. subsp. *culinaris*	Not available in public domain	do	
CDC Richlea	*L. culinaris* Medik. subsp. *culinaris*	Derived from a selection from the cross Laird/PI 179310.	do	Tahir et al. 2011
CDC Greenland	*L. culinaris* Medik. subsp. *culinaris*	Not available in public domain	do	
CDC LeMay	*L. culinaris* Medik. subsp. *culinaris*	CDC LeMay was selected from an F_2 derived family originating from Cross 983 between PI 486128 and FVR9Y-11. PI 486128 is the French cultivar Du Puy and FVR9Y-11 is a high-yielding CDC breeding line originally developed from the cross Du Puy × PI 345634.	do	Vandenberg et al. 2005
CDC Red Rider	*L. culinaris* Medik. subsp. *culinaris*	Not available in public domain	do	
CDC Maxim	*L. culinaris* Medik. subsp. *culinaris*	Not available in public domain	do	
CDC Rouleau	*L. culinaris* Medik. subsp. *culinaris*	Not available in public domain	do	

Table 5.1. Description of *Lens* genotypes used for genotyping with 39 SSR markers (continued).

Genotype	Species	Pedigree description	General description	Reference
CDC Viceroy	*L. culinaris* Medik. subsp. *culinaris*	Not available in public domain	do	
Riveland	*L. culinaris* Medik. subsp. *culinaris*	F5 selection from the cross of 'Laird'/ VW000412 (cross number X95L073).	Improved cultivar, maintained by Western Regional Plant Introduction Station, Pullman, USA.	McPhee and Muelbauher 2009
Pennell	*L. culinaris* Medik. subsp. *culinaris*	F_6 selection from the cross of LC660194/'Brewer'.	do	Muehlbauer and McPhee 2004
Eston	*L. culinaris* Medik. subsp. *culinaris*	Selection from PI 179307. This is an introduction from Turkey through the U.S. Department of Agriculture.	do	Slinkard and Bhatty 1981
Barimasur-4	*L. culinaris* Medik. subsp. *culinaris*	ILL588/FLIP-84-112L (ILL5782)	Improved cultivar in Bangladesh, maintained by the Western Regional Plant Introduction Station, Pullman, accession no PI 605356.	Sarker et al. 1999
IG 72618	*L. culinaris* subsp. *orientalis* (Boiss.) Penert	Germplasm	Wild germplasm collection from Turkey, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648625
IG 72896	*L. culinaris* subsp. *orientalis* (Boiss.) Penert	do	Wild germplasm collection from Uzbekistan, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648355

Table 5.1. Description of *Lens* genotypes used for genotyping with 39 SSR markers (continued).

Genotype	Species	Pedigree description	General description	Reference
IG 72594	*L. culinaris* subsp. *orientalis* (Boiss.) Penert	do	Wild germplasm collection from Iran, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648651
IG 72603	*L. culinaris* subsp. *orientalis* (Boiss.) Penert	do	Wild germplasm collection from Turkey, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648638
IG 72830	*L. culinaris* subsp. *tomentosus* (Ladiz.) M.E. Ferguson et al.	do	Wild germplasm collection from Turkey, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648421
IG 72688	*L. culinaris* subsp. *odemensis* (Ladiz.) M.E. Ferguson et al.	do	Wild germplasm collection from Syria, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648559
IG 110812	*L. lamottei* Czefranova	do	Wild germplasm collection from Spain, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648306
IG 72614	*L. culinaris* subsp. *tomentosus* (Ladiz.) M.E. Ferguson et al.	do	Germplasm collection from Turkey, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648628
IG 72616	*L. culinaris* subsp. *tomentosus* (Ladiz.) M.E. Ferguson et al.	do	Wild germplasm collection from Turkey, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648623

Table 5.1. Description of *Lens* genotypes used for genotyping with 39 SSR markers (continued).

Genotype	Species	Pedigree description	General description	Reference
IG 72549	*L. nigricans* (M. Bieb.) Webb & Berth	do	Wild germplasm collection from Croatia, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648692
IG 110810	*L. lamottei* Czefranova	do	Wild germplasm collection from Spain, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648307
PI 320937	*L. culinaris* Medik. subsp. *culinaris*	do	Germplasm collection from Germany, maintained by Western Regional Plant Introduction Station, Pullman, USA. Accession name is ILL 505.	https://www.genesys-pgr.org/acn/id/46329
WA8649090	*L. culinaris* Medik. subsp. *culinaris*	Pureline selection from bulk of 8 PI lines from Turkey (PI 370629 - 370636, consecutively).	Advanced breeding line, maintained by the Western Regional Plant Introduction Station, Pullman, USA.	Kahraman et al 2004a
Precoz	*L. culinaris* Medik. subsp. *culinaris*	Cultivar	Donated by Argentina, maintained by Western Regional Plant Introduction Station, Pullman, USA.	Kahraman et al 2004b
IG 572359	*L. nigricans* (M. Bieb.) Webb & Berth	do	Germplasm collection from Turkey, maintained by Western Regional Plant Introduction Station, Pullman, USA.	https://www.genesys-pgr.org/acn/id/92353
IG 110813	*L. lamottei* Czefranova	do	Wild germplasm collection from Spain, maintained by ICARDA, Syria.	https://www.genesys-pgr.org/acn/id/648303

Results

SSR genotyping

Of the 39 primers evaluated, 13 were polymorphic (Table 5.2) and the rest were monomorphic in 3% agarose gel. The polymorphism information content (PIC) of the polymorphic SSR markers ranged from 0.18 to 0.64 with an average value of 0.47. The number of alleles per locus ranged between 2 and 4 with an average of 3. The highest frequency of PIC value was observed between 0.41 and 0.50. Each SSR marker locus generated the expected band size with a range from 75 to 950 bp (Table A1). Out of the 13 polymorphic primers 6 amplified trinucleotide motifs, 1 with a tetra nucleotide motif, 1 with a penta nucleotide motif and 5 with dinucleotide motifs (Table 5.2).

Cluster and PCA analysis

The 13 polymorphic SSR markers identified 106 alleles in the 29 lentil genotypes. Jaccard similarity coefficient ranged between 0.31-0.72 (data not shown). The lowest similarity (0.31) was observed between the following pairs of genotypes: CDC Viceroy and CDC LeMay, WA8649090 and Eston, WA8649090 and CDC Viceroy (data not shown). The highest similarity (0.72) was observed between IG72616 and CDC Rouleau (data not shown). The Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) analysis grouped these genotypes into four major clusters (Fig. 5.1). PCA analysis of the SSR data resulted in clustering the 29 genotypes into four groups and distinct positioning of each genotype was observed within each group. The first three most informative components in the PCA analysis accounted for 37% (14, 12, and 11%, respectively) of the total variation. Genotypes in Cluster I were: CDC Maxim, CDC Rouleau, CDC LeMay, CDC Viceroy, CDC Rosetown, CDC Greenland, CDC Richlea, CDC Redberry, Barimasur-4, Eston, WA8649090, IG572359, Pennell, IG72549, IG72603, IG72688,

PI320937 or ILL505, Precoz, Riveland, IG72618 and IG72830. Cluster II consisted of IG72896 and IG72594. The genotypes in Cluster III were: CDC Red Rider, IG110810, IG110812, IG110813 and IG72614. Cluster IV was made of one genotype IG72616. Cluster I carried all cultivated types except CDC Red Rider which belonged to Cluster III.

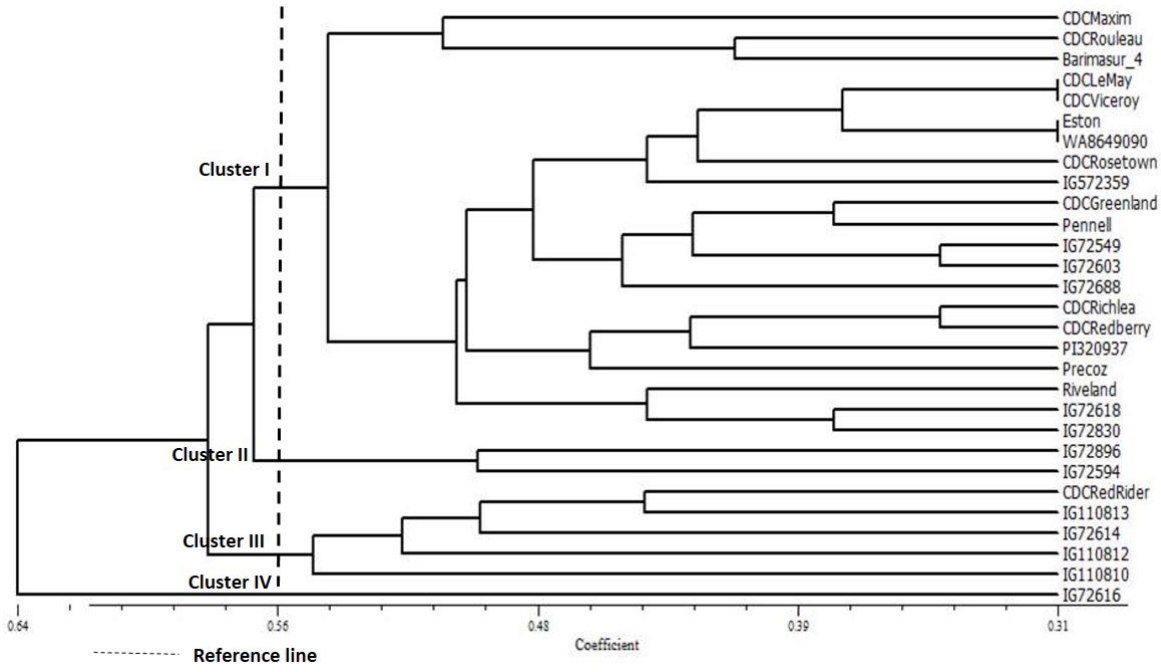

Fig. 5.1. Dendrogram showing genetic similarity among 29 *Lens* genotypes using 13 polymorphic SSRs based genotyping data. Cluster analysis was performed following un-weighted pair group method with arithmetic mean (UPGMA).

Table 5.2. Primer sequences of 13 polymorphic markers used in the genotyping of 29 *Lens* genotypes and their characteristics.

Primer ID	Forward primer (5'-3')	Reverse primer (5'-3')	Repeat motif	Alleles	PIC*
PBA_LC_0250	TGATTGATTCGGTACTTTTTG	ATGTTAATAAGCAGCAGCAAC	AAC	3	0.48
PBA_LC_0237	TGAAACCTTTTTGAAGACAAG	TCCATCTTCTAGATTCTTCCA	TAG	3	0.54
PBA_LC_0278	GACGCAGAAGATTAAGGAGAC	ATTCTGACCATAACCATTCCT	GAT	3	0.49
PBA_LC_0315	CTCTGAGCATCAATGAGTTTC	GGCACATTACTGTATGCATTT	GAG	4	0.60
PBA_LC_0323	GAATCAGTGTTCGTGTTCAAT	TTGAAGAAACCTGAAGATCAA	CGCAT	4	0.64
PBA_LC_0327	CCAAGAGCCATCAGAAATAG	AGGACTATCACGAAGAAAACC	GAA	4	0.62
PBA_LC_0369	AATGAGAGATATTCTTTGATTGG	GTGATAGGACTACATGGCAAA	TTCA	3	0.49
PBA_LC_0373	ATTTGGGCAACATATTCAAG	ACTATACTTTCTCCCGTCGTT	TCA	2	0.28
AC146588b	GGGTTCTATGCATTCTTCGC	CCTCCCTCCCTCTCTCTCTC	AT	3	0.45
AC146588c	CCTCCCTCCCTCTCTCTCTC	CCTCCCTCCCTCTCTCTCTC	AT	3	0.41
AC148097a	TTGGTGCACCGTATTTTGAG	CCAGGCATCCTTTTCTTTTC	AT	3	0.50
AC148097b	TTGGTGCACCGTATTTTGAG	CCAGGCATCCTTTTCTTTTC	AT	2	0.18
AC152551	TCAGCTTCATCAGCCAAAGA	CCAAACAGGGCCATAGACTC	AT	3	0.48

***** PIC denotes polymorphic information content.

Discussion

Several molecular marker systems have been used to evaluate lentil, e.g., random amplified polymorphic DNA (RAPD) (Abo-elwafa et al. 1995; Sharma et al. 1995; Ford et al. 1997; Rana et al. 2007), sequence tagged microsatellites (STMS) (Rana et al. 2007; Inder et al. 2008; Datta et al. 2011), SSR (Liu et al. 2008; Hamweigh et al. 2009; Babayeva et al. 2009; Reddy et al. 2010; Kaur et al. 2011, 2014; Verma et al. 2013, 2014), and inter-SSR (ISSR) and amplified fragment length polymorphism (AFLP) (Toklu et al. 2009). Among these, only a few studies (Abo-elwafa et al. 1995; Sharma et al. 1995; Hamweih et al. 2009; Reddy et al. 2010; Alo et al. 2011) included multiple *Lens* species. Clusters of genotypes is in agreement with the pedigree relationships (Table 5.1). Most of the *L. culinaris* subsp. *culinaris* genotypes were grouped together (Cluster I) (Fig. 5.1), and *L. culinaris* subsp. *orientalis* are in close proximity in Cluster II. Cluster III predominantly included *L. lamottei* genotypes along with one *L. culinaris* subsp. *culinaris* and one *L. culinaris* subsp. *tomentosus* genotype. Cluster IV included only one *L. culinaris* subsp. *tomentosus* genotype. The close proximity of *L. lamottei* and *L. culinaris* subsp. *tomentosus* genotypes are in agreement with earlier reports (Alo et al. 2011). The *L. nigricans* genotypes could not be distinguished and this is likely due to the limited number of polymorphic markers used. The *L. nigricans* genotypes clustered with *L. culinaris* types in Cluster I which does not agree with previously published relationship (Alo et al. 2011) and may be due to homoplasy (Cieslarová et al. 2011). The main morphological difference between *L. culinaris* and *L. culinaris* subsp. *orientalis* with *L. nigricans* is that of stipule shape; stipules for *L. culinaris* and *L. culinaris* subsp. *orientalis* are oblong or elliptic, lanceolate, whereas *L. nigricans* has one stipule that is semi-hestate, entire or dentate (Barulina 1930). Selection of genotypes based on genetic relatedness and phenotypic traits could be utilized to develop

intraspecific or interspecific mapping populations. Among the polymorphic markers, AC146588b, AC146588c, AC148097a, AC148097b, and AC152551 were previously mapped in an intraspecific lentil mapping population [cv. Northfield (ILL5588) × cv. Digger (ILL5722)]. Similarly, polymorphic markers; PBA_LC_0250, PBA_LC_0323, PBA_LC_0369, and PBA_LC_0373 were mapped in an intraspecific lentil mapping population (Cassab × ILL2024) that exhibits segregation for boron tolerance (Kaur et al. 2014). From the present study the allelic differences among the different lentil genotypes for the polymorphic loci are known now, which can be further utilized along with any phenotypic data for trait association with these markers. In summary, these polymorphic markers can be converted to functional markers for future pheonotypic studies.

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CHAPTER 6. DEVELOPMENT OF A PANEL OF UNIGENE DERIVED POLYMORPHIC EST-SSR MARKERS IN LENTIL USING PUBLIC DATABASE INFORMATION

Abstract

Lentil (*Lens culinaris* Medik.) is a diploid (2n=14) with a genome size of 4063 Mbp and is an important cool season food legume grown worldwide. Availability of genomic resources is limited in this crop species. The objective of this study was to develop polymorphic markers in lentil using publically curated expressed sequence tags (ESTs) information. In this study, 9513 expressed sequence tags (EST) were systematically downloaded from the National Center for Biotechnology Information (NCBI) database to develop unigene-based simple sequence repeat (SSR) markers. The ESTs were assembled into 4053 unigenes, and then analyzed to detect 373 SSRs using the microsatellite identification tool (MISA). Among the 373 SSRs, 26 compound SSRs were observed. Primer pairs for these SSRs were designed using Primer3 v1.14. To classify the functional annotation of ESTs, and EST-SSRs, BLASTx searches (E-value, $1x10^{-5}$) were performed against the publicly available UniProt (www.uniprot.org) and NCBI (www.ncbi.nlh.nih.gov) databases. Further functional annotation was performed using the PLAZA comparative genomics and GO annotation was slimmed using the Plant GOSlim category. Among the synthesized 312 primers, 219 successfully amplified *Lens* DNA. A diverse panel of twenty-two *Lens* genotypes were tested to identify polymorphic markers using 219 markers. A set of polymorphic 57 markers discriminated the *Lens* genotypes. This set of polymorphic markers with the functional annotation data are available as molecular tools to lentil breeding.

Introduction

Lentil (*Lens culinaris* Medik.) is a nutritious food legume crop grown throughout the world. Primary production regions include, Canada, Australia, Mid-western USA, Turkey, Syria and the Indian subcontinent (Nepal, India, Bangladesh). Annual world production is about 4.98 m tons (FAOSTAT 2013). USA produced 0.23 MT with average production of 1621 kg/ha (FAOSTAT 2013). US lentil is exported to India, Canada, Latin-America and the Middle-East. Small to medium seeded "Persian" type lentil were produced in USA followed by "Chilean" type (Yadav et al. 2007). Market classes of lentil include extra small red, small red, large red, small green, medium green, large green and French green.

Lentil originated in the Fertile Crescent (south west Asia and Mediterranean region) and believed to be one of the earliest domesticated food crops. The cultivated lentil, *Lens culinaris* has two types, *macrosperma* and *microsperma,* based on the seed and pod characteristics, length of the flowers, size of leaflets, length of vegetation and height of the plant (Barulina 1930). Similar to other food legumes lentil has a narrow genetic base. Realization of potential yield is limited due to various biotic and abiotic stresses like foliar and root diseases, high or low temperature, soil pH, and water logging. Optimization of crop management is also important, for example weed management or fertility which vary among growing environments. Breeding programs worldwide are working to breed for high yielding lentil cultivars with resistance to one or more of these stresses. Many breeding programs have implemented marker assisted breeding to speed up the selection process.

Availability of molecular markers and the ease of use in large breeding programs is a priority for many crop species. Number of available polymorphic markers is limited in lentil; and may be partly due to non-availability of a full genome sequence as well as the complexity of the

large genome (4063 Mbp) (Arumuganathan and Earle, 1991). Hamweigh et al. (2009) developed 14 microsatellite markers from a genomic library developed on lentil cultivar ILL5588. The genetic diversity index calculated based on the number of alleles amplified were reported to be high and markers were powerful enough to discriminate the test major groups, cultivated and wild types. In another study, Kaur et al. (2011) developed EST-SSR markers through transcriptome sequencing of lentil and validated 79 polymorphic EST-SSRs among 13 lentil genotypes including one *L. nigricans* accession. Verma et al. (2013) developed EST-SSRs through transcriptome sequencing of lentil genotype 'Precoz' (Buchwalt et al. 2004) and validated 54 polymorphic EST-SSRs among 22 lentil genotypes including one *L. culinaris* subsp. *orientalis* and two *L. lamottei* genotypes.

The total number of ESTs (9513) for lentil has remained constant in the National Center of Biological Information (NCBI) database. Development of genomic or transcriptome libraries are expensive and time consuming. Researchers working in various crop species like wheat (Gupta et al. 2013), chickpea (Choudhary et al. 2009), pea (Gong et al. 2010), *Medicago* (Gupta and Prasad 2009) have developed polymorphic markers utilizing sequence information available in public databases.

Use of genic SSR markers or EST-SSRs is more important from a breeding point of view. Despite recent advances in molecular marker systems like SNPs (Single Nucleotide Polymorphisms) or DNA array based marker systems SSRs hold promise as a breeder friendly marker system involving limited technical or operating difficulties. SSR markers are reproducible and PCR based resulting in easy application in breeding programs for marker assisted selection or predictions of breeding values. The availability of public databases like NCBI NR, UNIPROT, and TAIR help to further functionally annotate the ESTs or EST-SSRs.

This algorithm based or alignment based prediction of gene function could be verified in a trait specific case. The synchronization between functional annotation and wet lab validation largely depends on the standard of draft sequence available. Functional annotation of the SSRs provides opportunity for expression analysis of specific genes. The objectives of this study were to: (1) develop polymorphic SSR markers in lentil using EST sequences, (2) validate polymorphic EST-SSR markers within a diverse panel of *Lens* genotypes including wild lentil species, and (3) functionally annotate the EST-SSRs using publically available protein databases.

Materials and methods

EST sequences assembly, SSR detection and functional annotation

A curated search was performed in NCBI with query ("*Lens culinaris*"[Organism] OR *Lens culinaris* [All Fields]) AND "*Lens culinaris*"[porgn]) and 9,513 expressed sequence tags (ESTs) were systematically downloaded. ESTs representing "*Lens culinaris*/*Colletotrichum truncatum* mixed EST library"[porgn: __txid880151] were excluded and only *Lens culinaris* specific ESTs were further used for the downstream analysis. All the downloaded ESTs were cleaned for contamination using UniVec available from www.ncbi.nlm.nih.gov/tools/vecscreen/univec. Subsequently, cleaned ESTs were assembled using the Overlap-Layout-Consensus assembler MIRA (Mimicking Intelligent Read Assembly) (parameters: job = denovo, est, accurate, 454 using the -notraceinfo option) (Chevreux et al. 2004). Following the MIRA assembly, unigenes were created using the CAP3 (Huang and Madan 1999) with parameters –p 95, –o 49, and –t 10000 as previously implemented (Dubey et al. 2011; Zheng et al. 2011; Duvick et al. 2008). In addition to the parameters described in Zheng et al. (2011) the parameter -t value was extended to 10000 which improved the quality of the assembly using the maximum available memory, and avoiding the misassembly of the ESTs and

formation of counterfeit longer assemblies as previously suggested (Dubey et al. 2011; Duvick et al. 2008). Assembled unigenes were searched for SSRs using MISA (http://pgrc.ipk-gatersleben.de/misa/) (Thiel et al. 2003). For classifying true SSRs, we defined, a minimum repetitive stretch of 10 nucleotides as mono-, a consecutive stretch of 6 repeat units to be classified as di-, and a stretch of 5 repeat units for each tri-, tetra-, penta- and hexa-nucleotide stretches as simple sequence repeats (SSRs). To identify and classify the compound repeats, the minimum distance between two repetitive units was kept at ≤100 bp as previously suggested in MISA (Thiel et al. 2003). Open reading frames were extracted from the assembled unigenes using the extract ORF utility of the EMBOSS package available from http://emboss.sourceforge.net.

Database mining

Development of EST-SSRs and primer design

Following the identification of the SSRs, primer pairs were designed using Primer3 core version 1.1.4 available from http://primer3.sourceforge.net with primer pair parameters minimum and maximum amplicon size: 100-300 bp; primer size (minimum, optimum, maximum): 18-27 bp; primer Tm (minimum, optimum, maximum): 57-63°C; primer GC content: 30-70%; CG clamp: 0; maximum end stability: 250; maximum Tm difference: 2; maximum self-complementarity: 6; maximum 39 self-complementarity: 3; maximum Ns accepted: 0; maximum poly-X:5.

Functional annotation of unigenes and EST-SSRs

For classifying the functional annotation and gene ontology of the ESTs, and EST-SSRs, we performed BLASTx searches (E-value, $1x10^{-5}$) against the publicly available GenBank nr (www.ncbi.nlm.nih.gov), UniProt (www.uniprot.org), and TAIR10

(https://www.arabidopsis.org) databases. Additional functional annotation and gene ontology was obtained using FastAnnotator, which employs a four way classification approach utilizing Blast2GO and additional sequence homology searching by BLAST against NCBI nr, gene ontology (GO) term assignment with default annotation rule parameters, InterProScan (IPS) identification of functional motifs, merging of Blast-based and IPS-based GO annotations and augmentation by Annex (Götz et al. 2008), PRIAM and RPS BLAST (Ashburner et al. 2000; Chen et al. 2012). GO annotations so obtained were further analyzed using GO-SLIM (Plant) and functional GO-SLIM categories were defined.

Plant material and DNA extraction

Four *Lens* genotypes (3 *L. culinaris* and 1 *L. nigricans*) were used for initial screening of 312 primers. A diverse panel of twenty-two *Len*s genotypes, consisting of *L. culinaris* advanced breeding lines, parents of mapping populations, wild types and genotypes of *L. nigricans*, *L. culinaris* ssp. *orientalis*, *L. lamottei* was tested to identify polymorphic markers among those primers amplifying *Lens* DNA. DNA samples were extracted from individual plant leaf tissue when seedlings were 2 weeks old using the DNeasy® Plant Mini Kit (QIAgen). The DNA concentrations of the extracted samples were recorded using a Nanodrop 2000c spectrophotometer (Nanodrop, Wilmington, USA). The extracted DNA samples were diluted to a uniform concentration of 20 µg/µl for successful PCR amplification.

Table 6.1. Details of plant materials used for testing of 219 primer pairs.

Genotype	Species	Pedigree	Reference
LO56	*Lens culinaris* Medik. subsp. *orientalis* (Boiss.) Penert	RIL parent	Havey and Muehlbauer, 1989
WA8649041	*Lens culinaris* Medik.	Pureline selection from bulk of 8 PI lines from Turkey, RIL parent	Kahraman et al 2004a
ILL669	*Lens culinaris* Medik.	RIL parent	Kahraman et al 2004a
WA8649090	*Lens culinaris* Medik. subsp. *culinaris*	Pureline selection from bulk of 8 PI lines from Turkey, RIL parent	Kahraman et al 2004a
Precoz	Medik. subsp. *culinaris*	Cultivar, Donated from Argentina; Synonym = ILL 1405 RIL parent	Kahraman et al 2004b
Red Chief	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in USA; RIL parent PI 181886/PI 329171	Havey and Muehlbauer 1989
LO4	*Lens culinaris* subsp. *orientalis* (Boiss.) Penert	RIL parent	Havey and Muehlbauer 1989
Pennell	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in Northern Plains, F6 selection from the cross of LC660194/'Brewer'.	Muehlbauer and McPhee 2004
Brewer	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in USA; RIL Parent	Muehlbauer, 1987
Barimasur 4	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in Bangladesh. ILL588/FLIP-84-112L (ILL5782).	Sarker et al. 1999a
Emerald II	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in USA	Muehlbauer 1987
PI72618	*Lens culinaris* subsp. *orientalis* (Boiss.) Penert	Germplasm from Turkey.	https://www.genesys-pgr.org/acn/id/648625
Morton	Medik. subsp. *culinaris*	Cultivar in USA Autumn-sown, winter-hardy	Muehlbauer and McPhee 2007
Morena	Medik. subsp. *culinaris*	Cultivar in USA Pardina/PI 297754	Personal communication

Table 6.1. Details of plant materials used for testing of 219 primer pairs (continued).

Genotype	Species	Pedigree	Reference
PI320937 /ILL 505	*Lens culinaris* Medik. subsp. *culinaris*	Germplasm collected from Germany	https://www.genesys-pgr.org/acn/id/46329
Barimasur 2	Medik. subsp. *culinaris*	Cultivar in Bangladesh, cross between ILL4353/ILL353.	Sarker et al. 1999b
CDC Redberry	Medik. subsp. *culinaris*	Cross between 1049F3 / 819-5R. Line 1049F3 was derived from the cross 567-16/545-8. Line 819-5R was derived from the cross 86-360/(458-258G(458-122/C8L27-RC//Precoz)F_2)F_1.	Vandenberg et al. 2006
Barimasur 3	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in Bangladesh.	Sarker et al., 1999c
Pardina	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in USA	Personal communication
Shasta	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in USA. LC960027/3/PI 345635/'Palouse'//'Brewer'	Personal communication
Avondale	*Lens culinaris* Medik. subsp. *culinaris*	Cultivar in USA	Personal communication
Lens nigricans	*Lens nigricans* (M. Bieb.) Webb & Berth	Germplasm	Personal communication

PCR amplification

Three hundred and twelve primer pairs were synthesized from Europhin, USA and used in this study. The PCR reactions (25 µl volume) were conducted in a ABI 7500 (Applied Biosystems, Foster, CA, USA) thermocycler and each reaction comprised of 2.5 µl of Taq buffer (Promega, USA), 1.5 µl $MgCl_2$ (25 mM) (Promega, USA), 0.20 mM of each dNTP (Promega, USA), 0.50 mM of each primer (Europhin, USA), 0.25 µl of Hot Start Taq polymerase (Promega, USA) and 20 ng of template DNA. For initial screening of primers Touchdown PCRs

were performed following conditions using DNA from 4 lentil genotypes: 94ºC for 3 minutes, followed by 18 cycles of 94ºC for 50 s, 65-55ºC for 50 s, 72ºC for 50 s followed by 20 cycles of 94ºC for 50 s, 55ºC for 50 s and 72ºC for 50 s and final elongation step of 72ºC for 7 m. The PCR products were resolved in 2% agarose gels (molecular biology grade) (Sigma, USA) and bands were scored using gel documentation system. Primers amplifying *Lens* DNA were validated among a set of 22 diverse *Lens* genotypes following the PCR conditions in a ABI 7500 thermocylcer: 94ºC for 5 minutes, followed by 42 cycles of 94ºC for 1 m, 50ºC for 1 m, 72ºC for 1 m followed by a final elongation step of 72ºC for 5 m. Forward primers were tagged with M13 sequence (CACGACGTTGTAAAACGAC) at the 5 ' end. Four dyes were used to set up the multipliex PCR reactions. PCR products were separated using an ABI3730xl (Applied Biosystems, Foster, CA, USA) according to manufacturer instructions with the addition of the ABI GeneScan LIZ500 size standard and amplification product sizes were determined using the GeneMapper® v3.7 software (Applied Biosystems).

Results

Assembly and SSR detection

A set of 9513 EST were downloaded in FASTA file format from the NCBI and clustered them at identity of 0.95 into 4106 unigene sequences (Table 6.2). Then unigenes which are shorter than 100 bp were removed and also trimmed at the ends for the homopolymer as sequencing ESTs always gives a common problem of homopolymer due to the star and the falling activity of the DNA polymerase. MIRA assembly of 9513 EST sequences ultimately generated 4053 unigene sequences. Lists of unigene sequences for the polymorphic markers will be made available on the cool season food legume database (https://www.coolseasonfoodlegume.org/) (Appendix C).

Table 6.2. Summary of data mining of unigene sequences of *Lens culinaris*.

Parameter	Number
Total ESTs	9513
Total size of examined sequences (bases)	2574487
Total Unigene sequence	4053
Total SSR detected	374
Sequences with more than one SSR	32
Total compound SSR	26
Total ESTs with SSR	348

The total length of analyzed sequences was 2574487 bases (Table 6.2). MISA detected 373 SSR bearing EST sequences among these unigenes (Table 6.2). Out of these EST-SSRs there were 32 sequences with more than one SSR (Table 6.2). Also 26 compound SSRs were observed (Table 6.2). For further analysis 348 EST-SSRs were chosen. Using Primer3 primer pairs were designed for 348 EST-SSRs (Table A2). In addition to that, 658 primer pairs (Table A3) were designed based on the plantGDB assembly (version187a) of *Lens culinaris* [(http://www.plantgdb.org/download/download.php?dir=/Sequence/ESTcontig/Lens_culinaris/current_version)]. These were designed based on the detected EST-SSRs, which were further e-validated using ipcress *in silico* PCR amplifying software. In the validation experiment 312 primers were used here, among those 48 primers were from the e-validated list (Table A3) and remaining 264 were from Table A2. E-validated primers are coded with prefix "PUT" and other primers with "UN" (Table 6.3).

Structural and functional annotation of ESTs and EST-SSRs

Contig length ranged between 199-2599 bp (Fig. 6.1). The most prevalent contig length was 600-799 bp followed by 400-599 and 800-999, respectively (Fig. 6.1). After functional GO-SLIM analysis it was found that distribution of unigenes among GO, Domain and Enzyme

category was 54%, 42% and 4%, respectively (Fig. 6.2). This was consistent with the categorization of EST-SSRs into functional Go, Domain and Enzyme categories which were 56%, 40% and 4%, respectively (Fig. 6.3). In the GO category -Biological Process, the first four processes were oxidation-reduction process, ribosome biogenesis, translation and regulation of transcription, and DNA dependent for total number of unigenes analyzed (Fig. 6.4). Similar trend was observed using GO- Biological Process analysis of EST-SSRs where ranking of processes were as following, oxidation-reduction process, regulation of transcription, DNA dependent, ribosome biogenesis, and translation (Fig. 6.5). Total number of unigenes in GO category-molecular function showed the first four functions as DNA binding, nutrient reservoir activity, structural constituent of ribosome, and zinc ion binding (Fig. 6.6). However, for EST-SSRs, first four functions were, ATP binding, DNA binding, structural constituent of ribosome, and zinc ion binding in the GO category-molecular function (Fig. 6.7). In the GO category-Cellular Process, first four functions were for nucleus, cytosol, plasma membrane and chloroplast when the total number of unigenes were analyzed (Fig. 6.8). EST-SSRs analyzed for GO category-cellular process the first four functions were cytosol, plasma membrane, nucleus and integral to membrane (Fig. 6.9).

Frequency and distribution of EST-SSRs.

The frequency of SSRs was 6.9 per kb of sequence analyzed (373 SSRs/ 2575 kb of sequences) and there were 21 repeat patterns observed (Fig. 6.10). The most prevalent motif with greatest frequency was the trinucleotide followed by mono-, di-, tetra- and pentanucleotide repeat patterns. The highest number of repeat was observed in case of AG/CT, followed by AAG/CTT, AAC/GTT, ATC/ATG, AT/AT (Fig. 6.10).

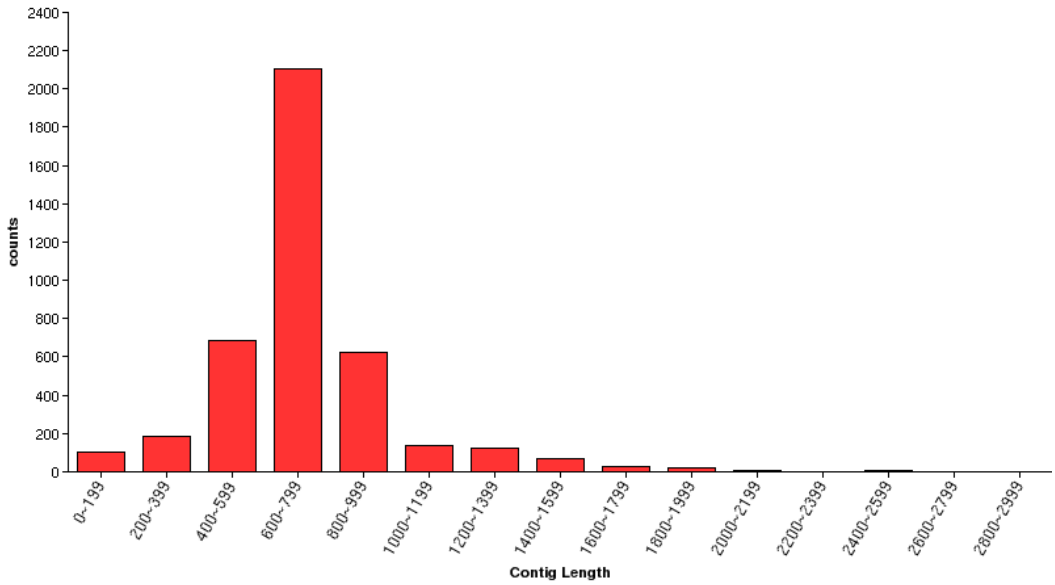

Fig. 6.1. Contig length distribution of the 4053 unigenes.

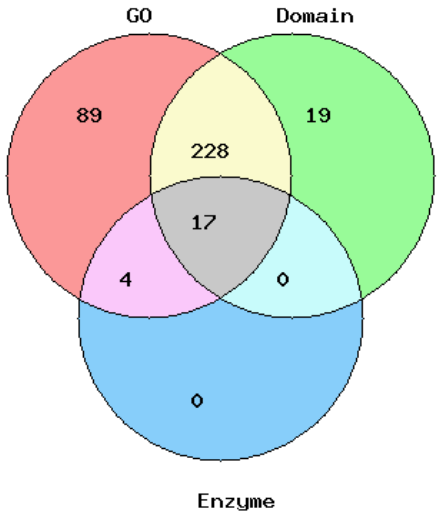

Fig. 6.2. Functional annotation of EST-SSRs.

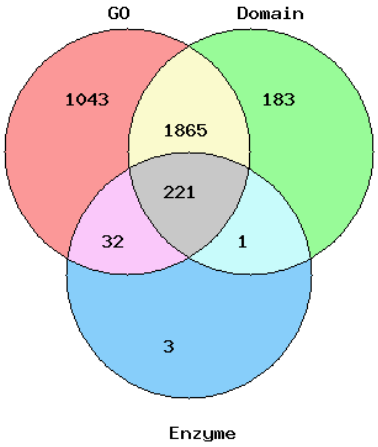

Fig. 6.3. Functional annotation of unigenes.

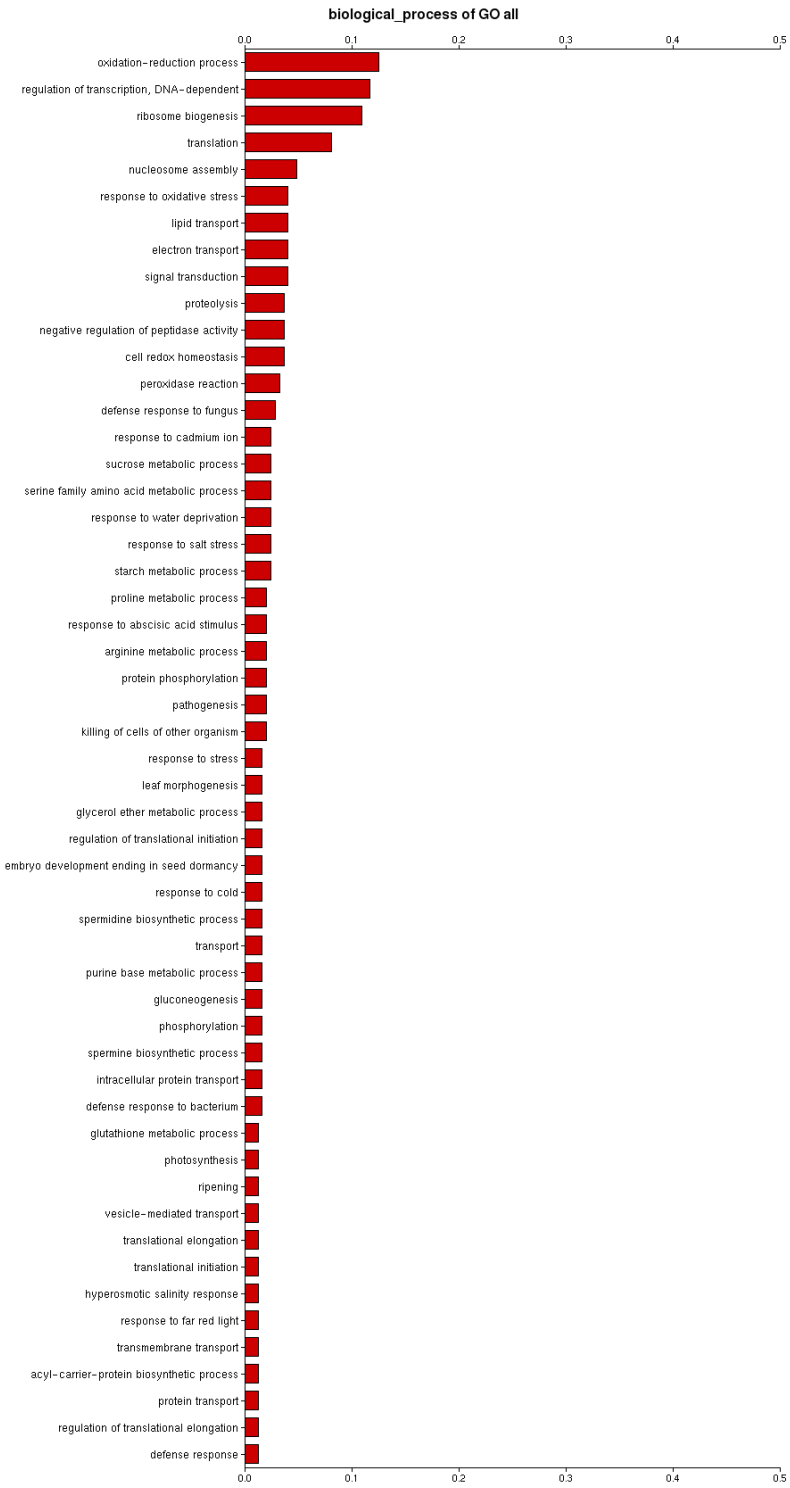

Fig. 6.4. GO category-Biological process annotation of 4053 unigenes.

Fig. 6.5. GO category-Biological process annotation of 373 EST-SSRs.

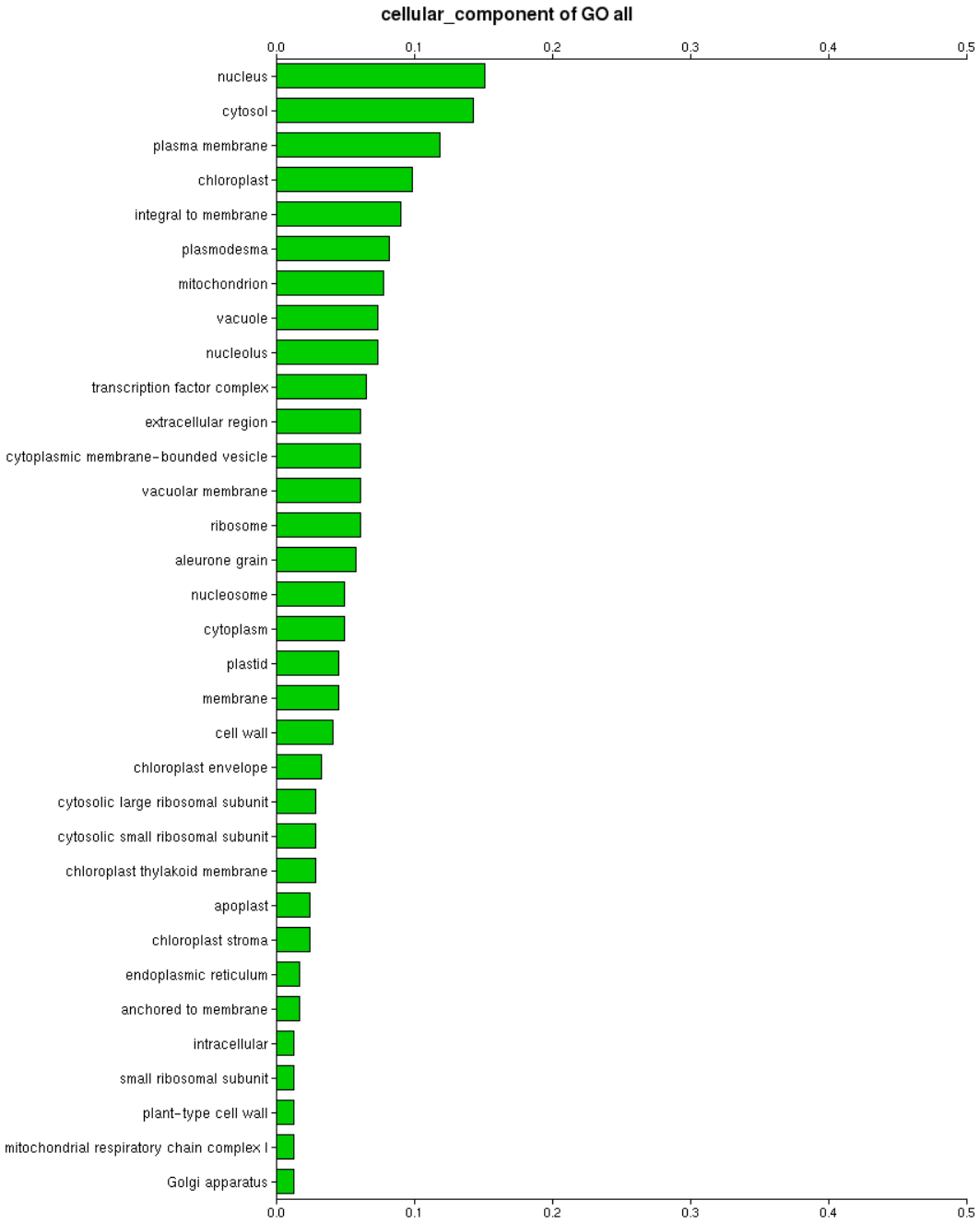

Fig. 6.6. GO category-cellular component annotation of 4053 unigenes.

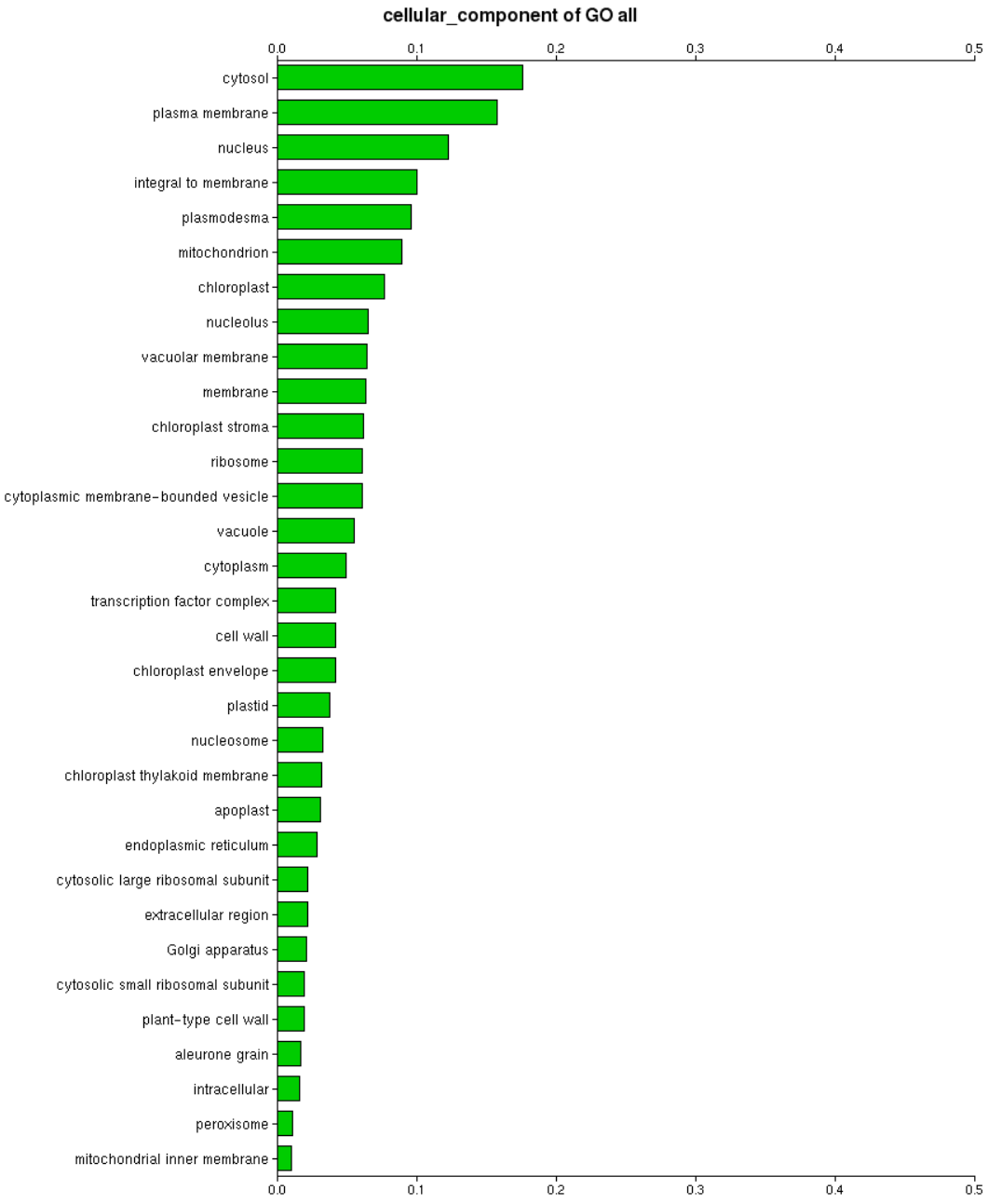

Fig. 6.7. GO category-cellular component annotation of 373 EST-SSRs.

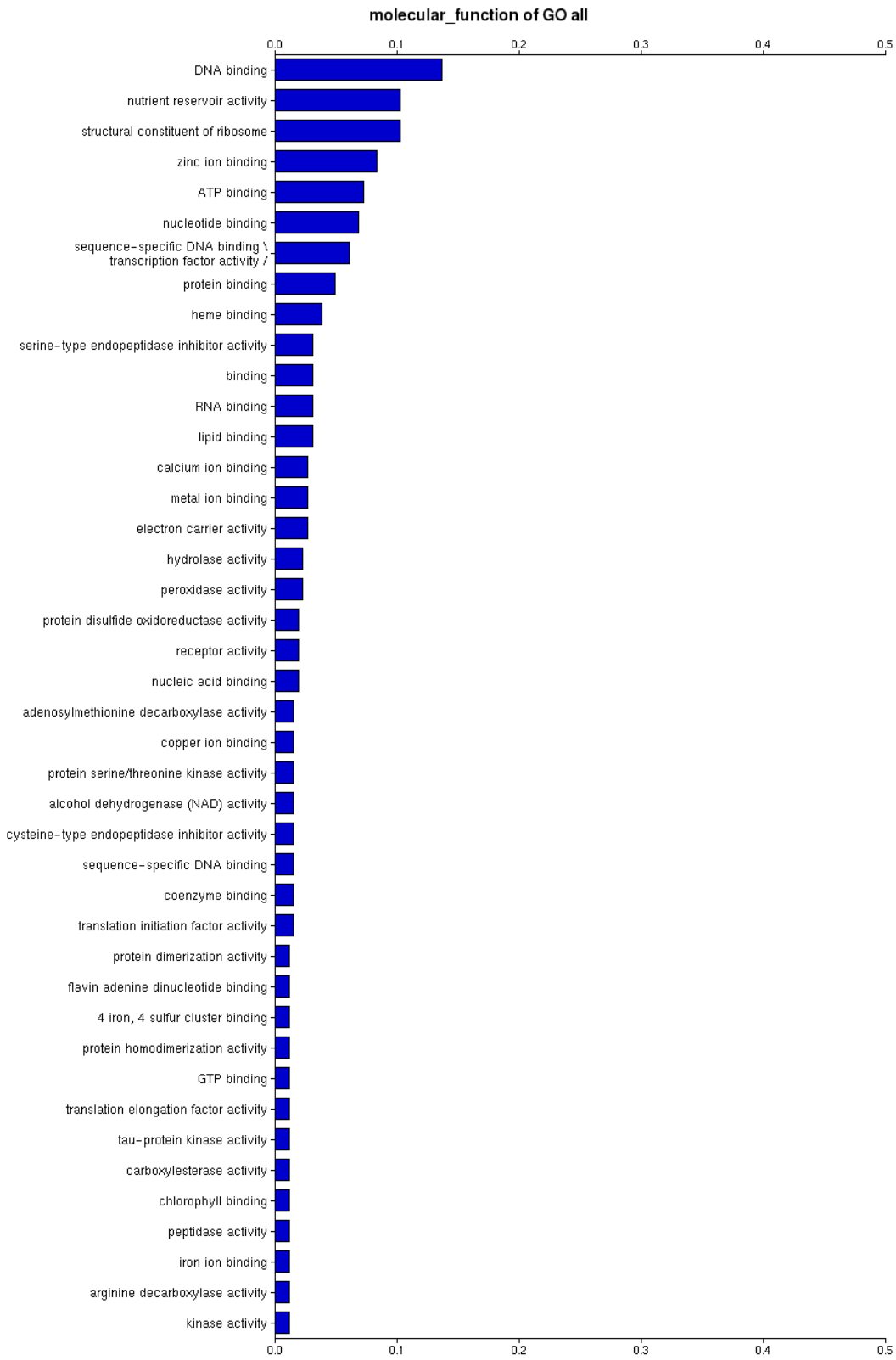

Fig. 6.8. GO category-molecular function annotation of 4053 unigenes.

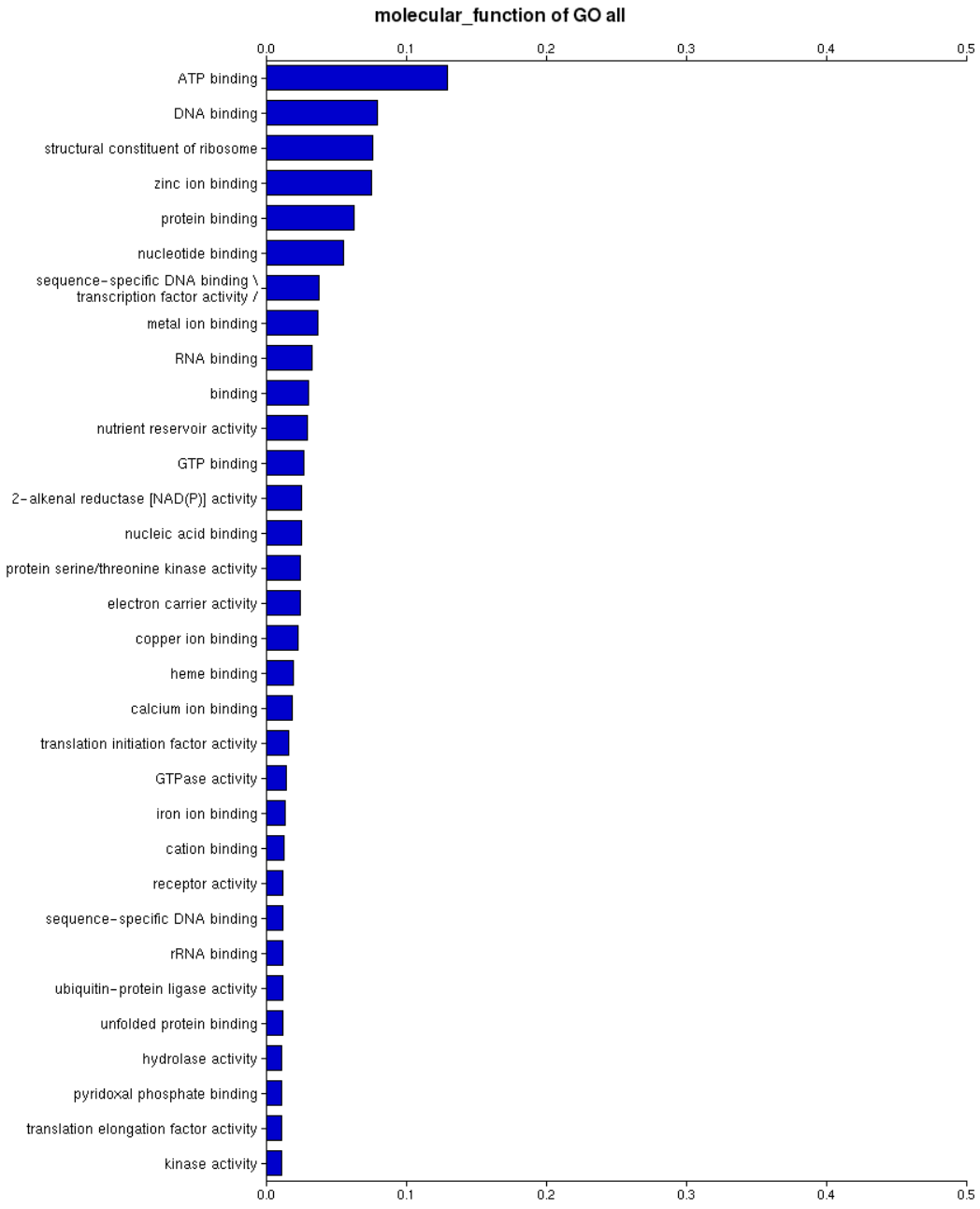

Fig. 6.9. GO category-molecular function annotation of 373 EST-SSRs.

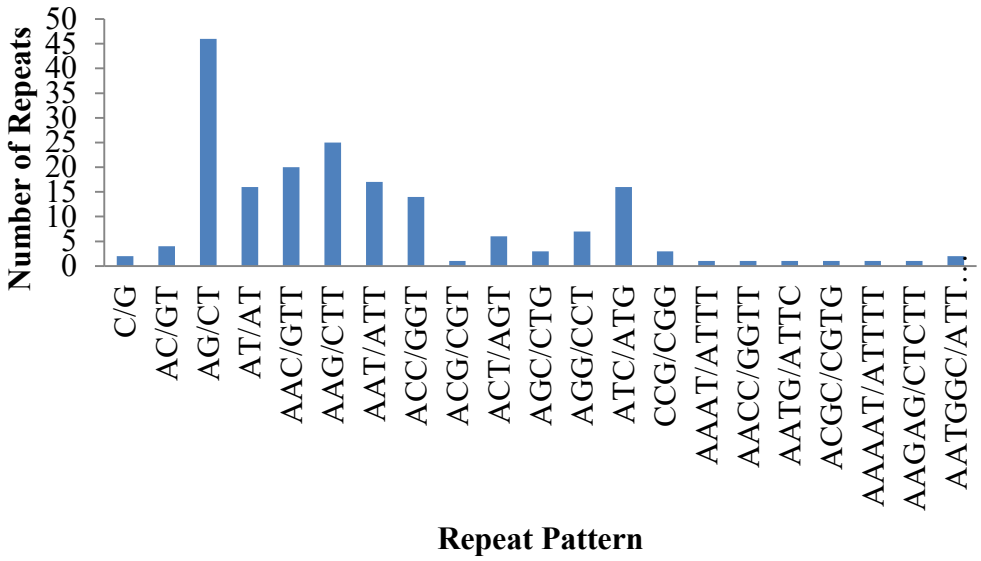

Fig. 6.10. Distribution of the simple sequence repeats across 4053 *Lens culinaris* unigenes.

Validation of EST-SSRs

Among the synthesized 312 primers, 219 successfully amplified *Lens* DNA. A diverse panel of 22 *Lens* genotypes, consisting of *L. culinaris* advanced breeding lines, parents of mapping populations, wild types and genotypes *of L. nigricans*, *L. culinaris ssp. orientalis*, *L. lamottei* was tested to identify polymorphic markers. A set of 57 polymorphic markers were found by testing 219 primers. The number of alleles amplified ranged between 2-17 for each primer and PIC ranged between 0.10-0.91. The average number of alleles produced per primer was seven.

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs.

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
PUT99	PUT187a Lensculinaris99	(AG)10	Histidine-containing phosphotransfer protein [*Medicago truncatula*]	GCGACCACTGTGTTGTTTGT	60	ATTTGAAGTCGGTGAGGTCG	60	316-322	0.65
PUT668	PUT187a Lensculinaris668	(AG)9	PHD1 protein [*Medicago truncatula*]	TTTTGCAGAGACGAGAGAGAAA	60	TCAGGATCGCATTGGTTGTA	60	147-149	0.40
PUT1105	PUT187a Lensculinaris1105	(TTG)6	unknown protein [*Medicago truncatula*]	AGGAGGAGGAGGATGTTGCT	60	CGCACTTCCAGACAAGTTCA	60	123-129	0.54
PUT1231	PUT187a Lensculinaris1231	(ACC)5	proline rich protein [*Medicago truncatula*]	TGTGGTACATGCACACCAAAT	60	GGTGGTAGCAGTGGTGGAGT	60	228-244	0.49
PUT1263	PUT187a Lensculinaris1263	(TGG)5	aspartic proteinase nepenthesin-2 [*Medicago truncatula*]	TCACTACCGGGAGAAAGTGG	60	CTACCCACCACCTCCTCAAA	60	130-136	0.10
PUT1271	PUT187a Lensculinaris1271	(AG)6	BEL1-like homeodomain protein [*Medicago truncatula*]	GGAGAGAAAGAGACGACAGGAG	60	TCGTTTTCTCTTCTGCGGTT	60	234-237	0.35

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
PUT2033	PUT187a Lensculinaris2033	(CCA)8	low-temperature inducible protein [*Medicago truncatula*]	ACAATCAGGTTTCGGACCAG	60	GCATCATCGATTTTGTGGTG	60	257-266	0.64
PUT2096	PUT187a Lensculinaris2096	(ATC)5	BHLH transcription factor [*Medicago truncatula*]	TTGCATGTATGAAACCGCAT	60	ATGGAGAAGCTAAGGGGGAA	60	267-288	0.50
PUT2104	PUT187a Lensculinaris2104	(AAC)5	chaperone protein DNAJ [*Medicago truncatula*]	ATTGCAGCCAGAGTGGAATC	60	AGAACGGCGTAAGCAGAAAA	60	195-201	0.37
PUT2213	PUT187a Lensculinaris2213	(AAC)5	unknown protein	CGACCTTCAGAAAGCTTGATTC	60	CAACGCAGACAACAACACAG	59	270-299	0.62
UN3.1	UN0003	(A)12	acyl carrier protein [*Medicago truncatula*]	TGTGTGTTTGGAGCAATGCT	59	GATGAGGACCTGGACCTCCT	60	198-204	0.37
UN32	UN0032	(AT)6	eukaryotic aspartyl protease family protein [*Medicago truncatula*]	TGTTGGTGCTGGTAAGATAGGT	59	CCCTAACCAGCCCAAAGCAT	60	272-276	0.51
UN33.1	UN0033	(A)10	early nodulin-like protein [*Medicago truncatula*	CCCAAGCCAACCATTTTTGC	59	GCATCAGGTTTGCCACCAAG	60	177-182	0.30

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN46	UN0046	(TTC)6	phospholipid hydroperoxide glutathione peroxidase [*Medicago truncatula*]	TCAACTCGC ATCCTCTTCA CA	59	TGATTGGGG GTTTGATGG GG	60	231-238	0.47
UN3776	UN3776	(TATT) 5	PHD finger alfin-like protein [*Medicago truncatula*]	TCCAGGTAA ACGAGAAGT TGAAGA	60	AGTGTGTGA ATTCGTGCC CA	60	125-313	0.96
UN3302	UN3302	(CCT)5	hypothetical protein MTR_2g010790 [*Medicago truncatula*]	TGGCACCAC CAAAGAGAC TC	60	TGGGGTTCG AGATTGGGG TA	60	114-266	0.90
UN3176	UN3176	(T)10	protein nuclear fusion defective 6, chloroplastic/mitochon drial isoform X1 [*Cicer arietinum*]	TTTGCTTTTA GGCCGCCAA G	60	TCCCAGAAT GAAGGGTTA ACCA	59	211-264	0.66
UN3814.1	UN3814	(A)11	cyclin [*Medicago truncatula*]	TCGGTAGCT GCTAGTGTC AC	59	CTTCCACCA CCACCTTGA CA	60	231-373	0.75
UN3814	UN3814	(T)13	cyclin [*Medicago truncatula*]	TTGTGCAGG GTCGACCTT AC	60	GTCGATGTC CCAGATCAG CC	60	234-315	0.78

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN3720	UN3720	(A)10	structural maintenance of chromosomes domain protein [*Medicago truncatula*]	CTCACTCAC CCGAGAAAC TCA	59	CTTCTGCGA CGCAATGCT TT	60	230-387	0.69
UN3519	UN3519	(T)10cc gtattgta tttttacat ccaactta attaaaaa tcctaaca aactaaa aagatatt tcaaaaat (A)10	UDP-D-glucuronate 4-epimerase [*Medicago truncatula*]	TCCCTTTTCT TCTTGACCG AGA	59	GTTCCGTTTA CGCATGCGA A	60	284-291	0.83
UN3311	UN3311	(GAT)6	hypothetical protein MTR_1g069440 [*Medicago truncatula*]	ACATGCCTG TGGTGGTTG AT	60	AGTGACACC ATTTTCAGG GTCA	60	290-305	0.79
UN3728	UN3728	(CAA) 5	DCD (development and cell death) domain protein [*Medicago truncatula*]	ACTCGTCCA CCAAAAATG AACG	60	GCACCACCA AACTTAACT CCC	59	233-295	0.87
UN3652	UN3652	(AAC) 5	growth-regulating factor-like protein [*Medicago truncatula*]	CCGTTCAAG AAAGCCTGT GG	59	TCCAGATGA TGCTGATGA CCT	59	231-362	0.73

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN3321	UN3321	(CAC)5	protein PHR1-LIKE 1-like isoform X1 [*Cicer arietinum*]	ACGACTCTG TTTCTTCCGC A	60	CCCTCCGGA AACTTCTTTG C	59	146-417	0.90
UN3548	UN3548	(A)19	unknown [*Medicago truncatula*]	GCGGTGGCA AACGTTAAG TA	59	AAGCAGAAC CGAGCCAAG TT	60	178-542	0.90
UN3414	UN3414	(TTC)6	myb-like transcription factor family protein [*Medicago truncatula*]	CTCCTTCCAT TTCTCTTTCT GCA	59	GACAAGGGT CAGCAAGGT GA	60	216-226	0.54
UN3326	UN3326	(A)10	uv radiation resistance-associated-like protein [*Medicago truncatula*]	GGAGTTTCA TGCGCCAAG TT	59	GGGCCCCGT CAAATGTAA CA	61	147-202	0.84
UN3849	UN3849	(AG)7	defender against cell death [*Medicago truncatula*]	GACGACTTC AGTTGAAAC AGCT	59	TACCTGAAG GAGAGCGGT GA	60	298-347	0.78
UN3573	UN3573	(GT)11	unknown [*Medicago truncatula*]	AGGCGTCCT TTGTATGCA CA	60	AACAGTCAA CATAAACAA CAGCGA	60	109-120	0.79

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN3291	UN3291	(CAAC)5	vacuolar proton-inorganic pyrophosphatase [*Medicago truncatula*]	CAACCCATG GTGGTCTCC TC	60	CACGCGGAA AAGATTCAG CC	60	227-242	0.68
UN0079.2	UN0079	(GGC) 5	insecticidal lentil peptide, partial [*subsp. culinaris*]	TCGGGTGAG ACCATTGTT CG	60	CAGACACCA CTTGTTGCTG C	60	282-297	0.85
UN0099	UN0099	(T)20	transmembrane protein, putative [*Medicago truncatula*]	TACTCATCG CCGTTGGTG TT	60	TCCTTAGTTT CAAAACAGC TTTCA	57	271-292	0.81
UN0106	UN0106	(ATA)6	xylose isomerase [*Medicago truncatula*]	AGAAAAGGG GAAGGGGGA GA	60	CTTCCTCCCG ATTCTCACC G	60	131-209	0.68
UN0110	UN0110	(T)11	heat shock protein [*Medicago truncatula*]	AAGCTGATG CTGACATGC CT	60	CCATAAAAG TATGCCCAA CTTGCA	60	240-243	0.40
UN0119	UN0119	(A)21	Spastin [*Medicago truncatula*]	ACATTTTGG TTGAAGTCT GCCT	59	AGCTGCCTT GCCTCATTTC T	60	147-399	0.88

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN0123	UN0123	(CT)53	NADH-quinone oxidoreductase subunit F [*Medicago truncatula*]	ACCGTCTGATTGAGCACAGT	59	TCCAAAGCCATCCAGTTCCC	60	142-288	0.91
UN0146	UN0146	(GAT)7	translational elongation factor 1-beta [*Medicago truncatula*]	TGACACCAAGGCCACTGAAG	60	AGTTTGGATGCGCCCCATAA	60	143-461	0.89
UN0225	UN0225	(A)29	40S ribosomal protein SA [*Medicago truncatula*]	ACATGTTGCAATGCTTTTAGCCT	60	TTCTTGCTTGGCGTTGAAGC	60	190-326	0.77
UN0230	UN0230	(T)10	light-harvesting complex I chlorophyll A/B-binding protein [*Medicago truncatula*]	AGAGGGCTCCAACTCTGTGA	60	ACGGGCCGAATAATCATGCA	60	169-179	0.67
UN0281	UN0281	(A)22	predicted: photosystem I subunit O [*Cicer arietinum*]	TGTCTGGCTTGAGCAGAAGA	59	TGTTGCCATAGCTTGCCTCA	60	120-250	0.80
UN0536	UN0536	(TA)6	cysteine proteinase inhibitor [*Medicago truncatula*]	ATAGGCCTGCTTGGACCCTA	60	ACAAAGGCAATTTCCAAACGT	57	114-123	0.63

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN0538	UN0538	(T)12	myb transcription factor [*Medicago truncatula*]	GCAAAGAGC TCGTGTGTG TT	59	AGCAGTTAG ATCACAGCT ACCA	59	130-178	0.82
UN0575	UN0575	(T)12	predicted: arabinogalactan peptide 16-like [*Cicer arietinum*]	CGCTCAATC TCCTTCCCCT G	60	CCTCCTCCG CGTTCTACA AA	60	139-433	0.88
UN0748	UN0748	(A)10	acylamino-acid-releasing enzyme [*Medicago truncatula*]	CATTGCTGC GTGGTTCAA CA	60	TCAAATATT CAGTGTCAT GTTCTACTT	57	120-240	0.82
UN0755	UN0755	(ACC)5	proline rich protein [*Medicago truncatula*]	CATGCACAC CAAATCCAC CA	59	TATCGGTGG CACGACAAC AA	60	146-148	0.32
UN0861	UN0861	(GAA) 10	peroxidase [*Medicago truncatula*]	ACAACACCA TGATGAGCC TTG	59	TGTGTCATC CATGGACCA CA	59	271-359	0.78
UN0931	UN0931	(A)16	snakin-1 [*Medicago truncatula*]	AGGGACAAG GAAAATGCC CT	59	AGCCCTGTA CATCACCCA AA	59	127-158	0.72

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN0953	UN0953	(A)11	legumin [*Medicago truncatula*]	ACCTCGCAG CCATGAGAT TC	60	GCTCTCGCG AATCTTTGC AG	60	204-211	0.67
UN0982	UN0982	(A)18	non-specific lipid-transfer protein 3 [*Lens culinaris*]	TGATGGTGC GGTTTCAAG GT	60	CCTACTCCC CCATCCAGG TT	60	206-421	0.77
UN1014	UN1014	(A)19	Histone H3 [*Medicago truncatula*]	AGCTACCTG GCTACCCAT TT	58	GGATTTGCG AGCGGTTTG TT	60	130-467	0.84
UN1128	UN1128	(A)10	predicted: membrane-anchored ubiquitin-fold protein 3 [*Cicer arietinum*]	CACCAACAA CAACAGCAG CA	60	CCAACTCCT CTTCCGGCA TT	60	313-325	0.38
UN1583	UN1583	(TAT)5	unknown protein	CTTCCCGAT CGTCGTATC GT	59	TCAATTTTCT GCATCATGA ACCT	57	177-319	0.41
UN1952	UN1952	(TAT)8	1-aminocyclopropane-1-carboxylate oxidase [*Medicago truncatula*]	AGGACAAGT GTTGGTGTG GG	60	CAGTTCTAA ATCACTGCA TCGCA	60	264-285	0.70

Table 6.3. Tm, allele size, polymorphism information content (PIC), SSR type, and sequences of each of the 57 polymorphic expressed sequenced tagged-simple sequence repeats (EST-SSRs) primer pairs (continued).

Marker	Transcript /Unigene I.D.	SSR type	Putative function	Forward primer (5'-3')	T_m	Reverse primer (5'-3')	T_m	Allele size	PIC
UN2594	UN2594	(A)11cataatagcatctattaaaacatacatgatggacaagcaatttctcaac(A)12	wound-responsive family protein [*Medicago truncatula*]	TTCTTCTTCTCAATTCAGATCAACTT	57	GTACCTAAGCTGCTGGGGTC	60	215-251	0.82
UN2787	UN2787	(CAC)7	adenylate kinase [*Medicago truncatula*]	GCTACAAAAAGCGCGTTTGC	60	TCATAACACGTAGCGGCTCC	60	105-211	0.49
UN2827	UN2827	(TAA)5	hypothetical protein MTR_1g084000 [*Medicago truncatula*]	AGCAGAAAGCACATTGCACA	59	CAAAGGCTGGGAAGGCAAAG	60	285-293	0.41

Discussion

MIRA assembly is flexible, allowing short reads like ESTs to be assembled into contigs and specific trimming further improved the quality of the sequences (http://mira-assembler.sourceforge.net/docs/DefinitiveGuideToMIRA.html). The number of SSR containing sequences detected was very high compared to other studies however it yielded a lower number of polymorphic markers. These polymorphic markers successfully discriminated the test genotypes and grouped genetically more related individuals. Simple sequence based markers are the most robust and easy to use marker systems. Moreover, capillary based gel separation technologies now help to detect small differences in length among the alleles. Polymorphic marker generated as many as seventeen alleles. Similar results were obtained in other crops where EST databases were used to develop SSR markers (Akash and Myers 2012; Gong et al. 2010; Gupta and Prasad 2009; Choudhary et al. 2009; Gupta et al. 2013). Kumar et al. (2015) reviewed the recent development of genic SSR markers in lentil (Kaur et al. 2011, 2014; Verma et al. 2013, 2014) and Andeden et al. (2015) developed 78 polymorphic SSR markers in lentil. However the number of polymorphic genic SSR markers were still limited. Kaur et al. (2011) validated a subset of 192 EST-SSR markers across a panel of 12 cultivated lentil genotypes which showed 47.5% polymorphism from a set of 2,393 EST-SSR markers. Kaur et al. (2014) found 40 polymorphic markers after testing 516 EST-SSRs. Andeden et al. (2015) developed (CA)n, (GA) n, (AAC)n and (ATG)n repeats enriched libraries and by sequencing these libraries found 78 polymorphic SSR markers using a set of 15 Turkish lentil genotypes. This study observed 21.6% polymorphism (out of the 360 primers validated 78 were polymorphic). In the present study 26% polymorphism was found by testing 219 markers in 22 cultivated and wild lentil genotypes. Verma et al. (2013) reported 42.59% polymorphism while validating 54

markers among 22 lentil and other genera, *Medicago*, *Glycine* and *Vigna*. The inclusion of other genera would have contributed to the higher polymorphism percentage. The use of SSR markers for diversity analysis or grouping of genotypes based on genetic relatedness in lentil or other closely related food legumes are reported by many workers (Wu et al. 2014; Kwon et al. 2012; Reddy et al. 2010; Liu et al. 2008). Kaur et al. 2011 and Verma et al. 2013 also found comparable grouping ability of the test polymorphic markers. The number of alleles amplified per locus was comparatively very low in case of Verma et al. (2013) (2.3 alleles) and Andeden et al. (2015) (5.1 alleles) compared to this study (7 alleles). Wong et al. (2015) classified four gene pools in lentil using genotyping-by-sequencing (GBS) of 60 genotypes. These were primary, secondary, tertiary and quaternary gene pools that were formed by *L. culinaris*/ *L. orientalis*/ *L. tomentosus*, *L. lamottei*/ *L. odemensis, L. ervoides,* respectively.

The distribution of functional annotation categories between the total ESTs and EST-SSRs were less comparable. It is noteworthy to mention that further SLIM-ing of GO categories improved the authenticity of the annotation data. It was observed that most of the functions under any annotation category remain the same between the unigenes and EST-SSRs. Functional annotations of the EST-SSR flanking regions indicated the involvement in the translating portion of the genome. This is important from the point of view of the development of functional markers in lentil. The lentil genome sequencing project is underway with the most recent draft (v 0.7) with approximately 150X coverage produced scaffolds covering about half of the genome. The initial assembly resulted in useful SNPs suitable for marker assisted selection (Bett et al. 2015). Development of dense genetic maps is a prerequisite in lentil (Sharpe et al. 2013) and recently EST-SSR and SNPs were mapped in lentil (Gupta et al. 2012; Kaur et al. 2014).

In conclusion, a polymorphic set of 57 markers were developed in lentils. Out of these 14 amplify the identical SSRs reported by Kaur et al. (2011). These were further validated among diverse lentil genotypes. These markers could be used by the lentil research community for molecular breeding.

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CHAPTER 7. DEVELOPMENT OF MOLECULAR MARKERS FOR IRON METABOLISM RELATED GENES IN LENTIL AND THEIR EXPRESSION ANALYSIS UNDER EXCESS IRON

Abstract

Multiple genes and transcription factors are involved in the uptake and translocation of iron in plants from soil. The sequence information about iron uptake and translocation related genes is largely unkown in lentil (*Lens culinaris* Medik). This study was designed to develop iron metabolism related molecular markers for *Ferritin-1*, *BHLH-1* (Basic helix loop helix) or FER-like transcription factor protein and *IRT-1* (Iron related transporter) genes using genome synteny with barrel medic (*Medicago truncatula*). The second objective of this study was to analyze differential gene expression under excess iron conditions over time (2h, 8h, 24h). Specific molecular markers were developed for iron metabolism related genes (*Ferritin-1, BHLH-1, IRT-1*) and validated in lentil. Gene specific markers for *Ferritin-1* and *IRT-1* were used for quantitative PCR (qPCR) studies based on their amplification efficiency. Significant differential expression of *Ferritin-1* and *IRT-1* was observed under excess iron conditions through qPCR based gene expression analysis. Regulation of iron uptake and translocation in lentil needs further characterization. Greater emphasis should be given to development of conditions simulating field conditions under external iron supply and considering adult plant physiology.

Introduction

Iron (Fe) uptake in plants is a complex physiological process governed by homeostatic mechanisms in the plant. Homeostatic mechanisms involve absorption, translocation and redistribution of Fe within the plant system at a particular concentration (10^{-9}-10^{-4} mol/l)

(Romheld and Scaaf 2004). Lower iron concentration leads to Fe-deficiency symptoms including chlorosis and necrosis in leaves and ultimately loss in biomass as well as grain yield. Higher concentrations of Fe within the plant system results in generation of free radical species which damage various cellular components by interacting with protein, lipid, carbohydrates and even with DNA. According to Welch and Graham (2004), there are four different barriers controlling homeostatic mechanisms of mineral uptake in plants; (A) the root-soil interphase known as rhizosphere, (B) root-cell plasma membrane, (C) translocation to edible plant organs (grains/tubers), and (D) bioavailability of minerals.

Ferritin is an iron-carrying protein in plants and has a multimeric (24-mer) cage-like structure that carries up to 4500 atoms of Fe within its core (Crichton et al. 1978; Wade et al. 1993). The ferritin protein is highly conserved within the animal and plant kingdom (Ragland et al. 1990). Ferritin meets the metabolic need for iron when required by the metabolome as well as prevents any kind of oxidative stress (Raymond and Bryan 1995; Waldo and Theil 1996; Harrison et al. 1998). Plant ferritin subunit sequences share between 39% and 49% similarity with mammalian ferritin sequences (Briat et al. 2009). This similarity increases when comparisons are made within the plant kingdom or among close plant families. Iron homeostasis is important due to the minute balance that exists between iron deficiency and toxicity and that affects plant physiology. Impaired plant physiology ultimately affects crop yield. Ferritin regulates iron homeostasis to prevent interaction of iron with other cellular components which may result in generation of free radicals during oxidative stress. In plants, ferritin consists of a single kind of subunit and ferritin bound Fe is highly bioavailable (Kalgaonkar and Lonnerdal 2008).

Lentil (*Lens culinaris* Medik.) being a dicot plant uses strategy I where ferric iron is reduced at the rhizosphere and absorbed as ferrous iron by the root. Monocot plants use a different strategy to uptake iron from the soil (strategy II). In *Arabidopsis thaliana*, reduction of ferric Fe is accomplished by Fe reductase FRO2 (ferric reductase oxidase-2; Robinson et al. 1999). This was the first report of cloning and gene function elucidation of any major iron metabolism related gene in plants. Uptake of ferrous Fe into the root is carried out by the metal transporter IRT1 (iron-regulated transporter; Eide et al. 1996; Vert et al. 2002). The basic helix-loop-helix (BHLH) transcription factor family in plants is a ubiquitous regulator and is highly conserved, regulating different types of genes during transcription (Heim et al. 2003). The BHLH transcription factor or FIT (FER-like Fe deficiency-induced transcription factor) is reported to be responsible for high-level expression of FRO2 and IRT-1 (Colangelo and Guerinot 2004; Jakoby et al. 2004; Yuan et al. 2005).

Development of gene specific markers and their utilization in understanding metabolic pathways are important genomic goals to achieve in any crop species for their effective utilization in genetic studies or molecular breeding applications *per se*. Availability of specific DNA markers for iron metabolism related genes in lentil are not available. The objectives of the study were to, (1) develop gene (*Ferritin-1*, *BHLH-1*, and *IRT-1*) specific molecular markers in lentil and (2) analyze their gene expression under excess iron over time.

Materials and methods

Plant materials and treatments

CDC Redberry (Vandenberg et al. 2006) seedlings were raised in the laboratory and fresh tissue was collected for DNA and RNA extraction. For gene expression analysis, CDC Redberry seeds were germinated on wet filter paper in an incubator maintained at 25°C. Seedlings were

transferred to hydroponic growth in 50 mL tubes containing distilled water and kept under growing conditions of 16 h of light and at 25°C for eight days after germination. After complete development of the first trifoliolate leaf (18–21 days of growth), two different treatments were made: (1) control with distilled water (iron deficient condition), (2) induction of excess iron condition by addition of 500 µM of Fe-EDTA, 150 mM of sodium citrate and 75 µM $FeSO_4$ (Lobre´aux et al. 1995). Treatments were applied for 24 h and samples were collected 2, 8 and 24 h after treatment. Three biological replications were included for each treatment.

Development of markers

Full length coding sequences (CDS) for three ferritin genes (*ferritin-1*, *ferritin-2*, *ferritin-3*) for *Medicago truncatula* were acquired from the NCBI (National Center for Biological Information) nucleotide database on 15 April 2015. We downloaded the complete coding sequence of *Ferritin-2* mRNA (NCBI reference sequence: XM_003616637.1) of *M. truncatula* in FASTA format and performed a nucleotide BLAST search against CDC Redberry 454 contig sequences in the Knowpulse database (http://www.knowpulse.usask.ca/portal/blast/nucleotide/nucleotide). The contig sequence with the highest bit score and lowest e-value and, therefore, having the highest similarity with the query sequence (*M. truncatula Ferritin-2*) was identified. Then the contig sequence was downloaded from the Knowpulse database and used to design primers. Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to design five primer pairs using default parameters (Table 7.1). One primer pair (FerrClo5) with reproducible and clear amplification was chosen for further analysis and development of qPCR compatible primers for the Ferritin gene in lentil. In addition, one primer pair specific to a lentil BHLH (Basic Helix Loop Helix) transcription factor or FER-like Transcription Factor gene sequence was

synthesized. Primers were also designed for the iron-related transporter gene based on the *IRT1* mRNA coding sequence (CDS) (LegumeIP database reference no. IMGA[Medtr8g105030.1] of *M. truncatula* for the amplification of lentil *IRT-1* in the qPCR experiment. The amplicon of Ferritin as well as the BHLH transcription factor gene were beyond the range of optimum product size (>250 bp) for qPCR experiment and thus were gel purified using a gel purification kit (IBI, MIDSCI, St. Louis, USA) (Vogelstein and Gillespie, 1979) following manufacturer's instructions and sequenced using the Sanger sequencing method (Etonbiosciences Inc., San Diego, CA). The gene sequences were aligned with the respective *M. truncatula mRNA* sequences (*Ferritin-2* and *BHLH* transcription factor gene, respectively) and primer pairs were designed for qPCR experiments based on the putative exonic sequences, their sequence identity, gap and the desired product size using Primer3 software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Based on these sequences one primer pair for *Ferritin-1* and another primer pair for *BHLH-1* transcription factor were designed for qPCR. Primers for *IRT-1* were directly used in qPCR and were within the qPCR compatible product size range (<100 bp amplicon size).

Isolation of RNA and synthesis of complementary DNA

Total RNA was extracted from 100 mg of fresh leaves of individual treatments using the QIAGEN® RNeasy Mini Kit (QIAGEN, California, USA) according to manufacturer instructions. The quality of the RNA extracts were determined by the spectrophotometer Nano-Drop (ND-1000) (NanoDrop Technologies, Welmington, USA). To check the integrity of the RNA, the samples were stained, separated and visualized by electrophoresis in a 1 % agarose gel. Details about the quality of the RNA samples can be found in Table A4. The first strand of cDNA was synthesized from 1 µg of total RNA in a 20 µL reaction using SuperScript III First

Strand Synthesis Supermix RT-PCR Kit (Invitrogen, USA). The cDNAs were diluted to 2 ng μL^{-1}.

Quantitative PCR

Three primer pairs were used for gene expression analysis, *Ferritin1* (developed using PCR based cloning and sequencing), *BHLH1* (developed using PCR based cloning and sequencing) and *IRT1* (primer designed based on *M. truncatula IRT1* gene sequence). Expression levels of mRNA were evaluated in a SYBR Green dye using an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, USA). PCR amplifications were carried out in triplicate in 20 µL reactions containing Maxima SYBR Green mixer (Fermentas, USA), 250 nM of each primer and 4 ng of cDNA. On each plate, the reference genes (*GADPH* and *Actin*) and negative controls were included. Amplification conditions were 50 °C for 2 min, 95 °C for 10 min, 40 cycles at 95 °C for 15 s, 60 °C for 1 min. The calibration curves for each primer pair were plotted using five serial dilutions of the cDNA in water. To verify the specificity of amplification a dissociation curve analysis step was added to the qPCR amplification protocol. Amplification efficiency, slope and R^2 value were determined for each primer pair. Amplification efficiencies were calculated by $E = (10^{-1/slope} - 1) \times 100$.

Statistical analysis of gene expression analysis

Cycle threshold (C_T) values were determined using SDS software (Applied Biosystems, USA). Gene expression data were analyzed using the C_T values and amplification efficiency values using method $2^{-\Delta\Delta CT}$ (Livak et al. 2001). Geometric means of reference genes were used to normalize the C_T values of the individual samples. The program REST 2009—Relative Expression Software Tool (Pfaffl 2001) was used to determine if the differences between the treatments were statistically significant ($P<0.05$).

Results

Development of markers

After performing BLASTn analysis using *ferritin-2* mRNA sequence of *Medicago truncatula* in the KnowPulse database (University of Saskatchewan, Canada) one contig sequence was identified, LcRBContig00605, based on BIT score (700), sequence identity (91%) and e-value (0) (Table 7.1). BLASTn results using other plant species resulted into the identification of this contig sequence (LcRBContig00605) (data not shown). Five primer pairs were designed using Primer-BLAST, *FerrClo1*, *FerrClo2*, *FerrClo3*, *FerrClo4*, and *FerrClo5* (Table 7.2). Among the five primer pairs *FerrClo5* produced reproducible and clear amplification of CDC Redberry genomic DNA. Optimum PCR conditions for *FerrClo5* primers in a ABI 7500 thermocylcer were established : 94ºC for 5 minutes, followed by 30 cycles of 94ºC for 1 m, 60ºC for 1 m, 72ºC for 1 m followed by a final elongation step of 72ºC for 5 m. The amplified DNA fragment was gel purified and sequenced using Sanger's method to obtain a 390 bases long sequence. The sequence has been submitted to National Center of Biological Information (NCBI) database. Alignment of the partial genomic DNA sequence with the *M.*

Table 7.1. Nucleotide BLAST results of *M. truncatula ferritin-2* gene sequence (NCBI reference no. XM_003616637.1) with CDC Redberry 454 contig sequences in Knowpulse database showing bit score, percent identity and e-value (http://knowpulse.usask.ca).

Hit*	Bit Score	Identity%	E-value
LcRBContig00605	700	91	0.00e+0
LcRBContig02360	530	90	1.53e-103
LcRBContig20139	142	93	4.44e-5
LcRBContig24460	167	94	1.39e-40
LcRBContig24460	167	94	1.39e-40
LcRBContig13391	167	94	1.39e-40
LcRBContig07868	167	94	1.39e-40
LcRBContig07177	167	94	1.39e-40
LcRBContig01318	167	94	1.39e-40
LcRBContig24151	111	91	7.13e-24

*First 10 relevant hits are shown here

truncatula ferritin-2 mRNA sequence (NCBI reference sequence: XM_003616637.1) showed a 92 bp sequence overlap with no gap (Fig. 7.1). This potential exonic sequence was used to design primers (*Ferritin-1*) using Primer-BLAST.

Primer pairs developed in a previous study were used to amplify the *BHLH-1* gene in CDC Redberry genomic DNA. Optimum PCR conditions for *BHLH-1* primer pairs in a ABI 7500 thermocylcer were established: 94ºC for 5 minutes, followed by 30 cycles of 94ºC for 1 m, 60ºC for 1 m, 72ºC for 1 m followed by a final elongation step of 72ºC for 5 m. The amplified fragment sequenced by Sanger's sequencing method and A 490 base long sequence for *BHLH-1* was submitted to the NCBI database. This sequence was aligned with *M. truncatula BHLH* mRNA sequence (NCBI reference number XM_003606283.1) and based on the alignment (Fig. 7.2) a 75 bp sequence with no gap (potential exonic sequence) was used to design qPCR compatible primers for *BHLH-1* in lentil using Primer-BLAST.

Table 7.2 Sequence information and T_m (melting temperature) of primers designed based on the CDC Redberry contig (LcRBContig00605) to clone *Ferritin-1* gene in lentil.

Primer name	Forward sequence(5'-3')	Reverse sequence(5'-3')	T_m (°C)
FerrClo1	TGCTGATAAGGGTGATGCGCT	GGCTTCCACCTGTTCACCCA	64
FerrClo2	AACCTGCACAGTGTTGCCTC	AGTGCCAGACACCATGTCCT	62
FerrClo3	GTTGCGCTGAAAGGTCTTGCT	GCCAAGTGCACATCACCAGT	62
FerrClo4	ACGTTGCGCTGAAAGGTCTTG	TGCCAAGTGCACATCACCAG	62
FerrClo5	CTGGTGATGTGCACTTGGCA	GCTGCAGCTGCTTCCTCACT	62

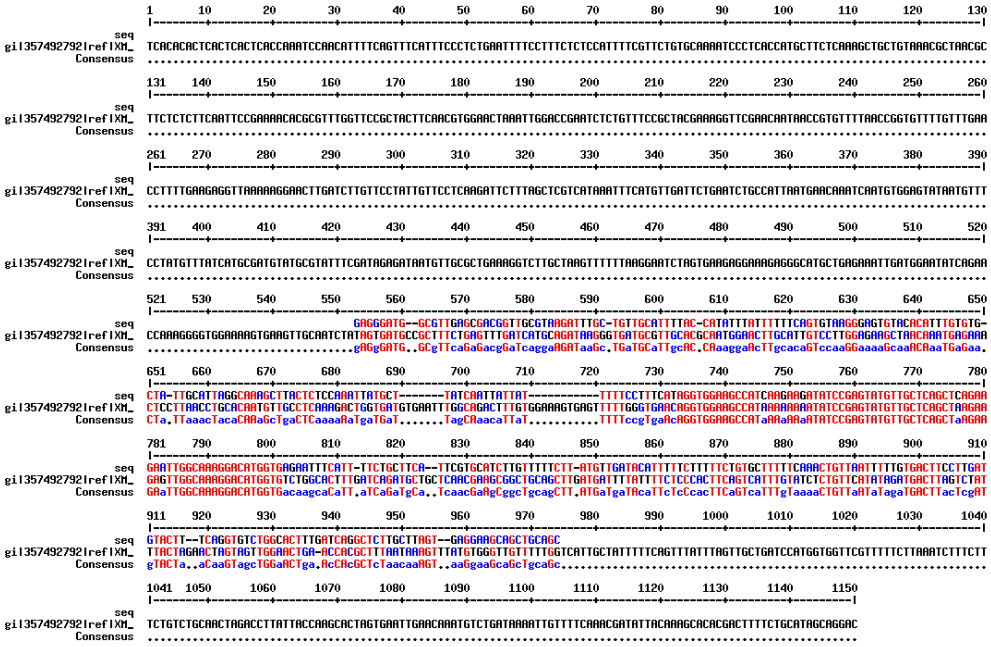

Fig. 7.1. Sequence alignment between *Medicago truncatula ferritin-2* full length CDS (NCBI reference no. XM_003616637.1) and lentil *Ferritin-1* partial genomic sequence using MultAlin tool (Corpet et al. 1988) with default parameter values. From 721 to 812 was used to design qPCR compatible primers.

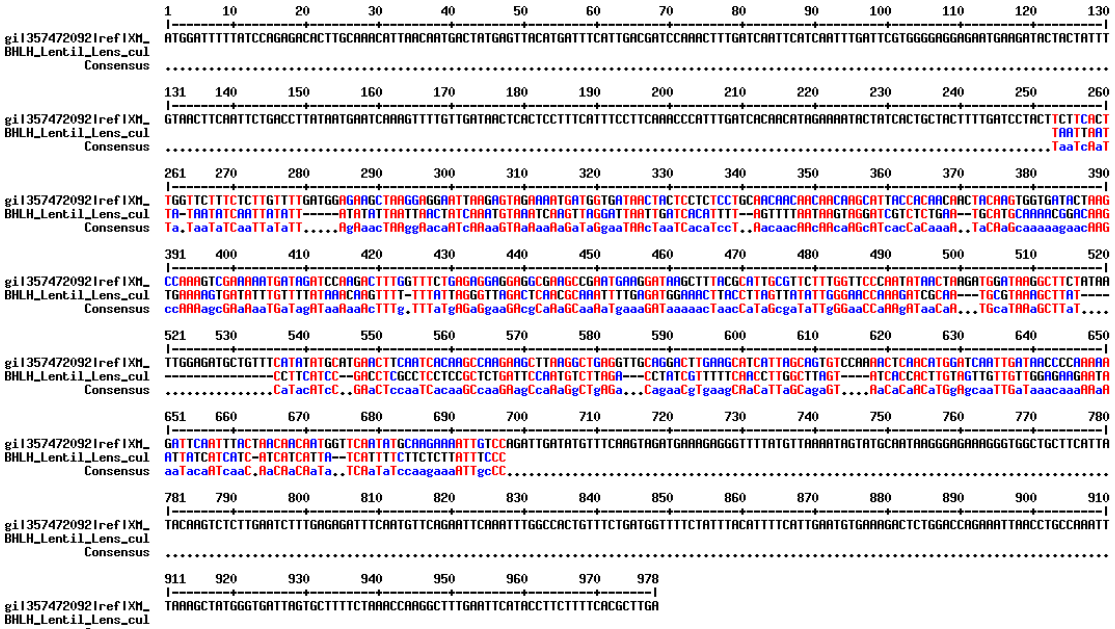

Fig. 7.2. Sequence alignment between *Medicago truncatula BHLH* full length CDS (NCBI reference number XM_003606283.1) and lentil *BHLH-1* partial genomic sequence using MultAlin (Corpet et al. 1988) with default parameter values. From 425 to 449 was used to design qPCR compatible primers.

Using a *M. truncatula* iron regulated transporter gene mRNA sequence [LegumeIP database reference no. IMGA(Medtr8g105030.1)] primer pairs (IRT1) were designed for the qPCR study. Dissociation curve analysis of the three pairs of primers (*Ferritin-1*, *BHLH-1*, *IRT-1*) showed specific amplification (Figs. 7.3, 7.4, 7.5). Amplification efficiency of the designed primer pairs and reference genes (*GADPH, Actin*) were found to be >90% with the exception of *BHLH-1* primer pairs (Table 7.3). Slope values ranged from -0.02 to -3.55 and R^2 values ranged between 0.0034 and 0.9972.

Table 7.3. Amplification statistics for one *Ferritin-1*, one *BHLH-1*, one *IRT-1* gene specific primer pairs, and one primer pair for each reference gene (*GADPH, Actin*).

Gene	Forward sequence	Reverse sequence	T_m (°C)	Size	Slope	R^2	E
Ferritin-1	AGATATCCG AGTATGTTG CTCAG	AAGATG CACGAA TGAAGC AGAAA	61	84	-3.32	0.9968	100.07
IRT-1	GTCGCTGT TTTGCTAG GTGC	GTGAGC TTCTCCT CTTCCCT	61	159	-3.12	0.9954	109.18
BHLH-1	TTATTAGG GTTAGACT CAACGCA	TTGCGAT CTTTGGT TCCCA	59	74	-0.02	0.0034	6.55e+ 42
GADPH	TGGGCGAA AACTCCAC TTTG	GAATTG CTGCAG CCTTGTG A	60	57	-3.15	0.9954	107.71
Actin	CCAAATCA TGTTTGAG GCTTTTAA	GTGAAA GAACGG CCTGAAT AGC	60	64	-3.55	0.9972	91.25

Here, T_m=melting temperature, Size=amplicon length, Slope=slope of the trend line in amplification efficiency graph, R^2=regression coefficient, E=amplification efficiency

Table 7.4. Differentially expressed *Ferritin-1* and *IRT-1* genes in CDC Redberry shoot and root tissues over time (2, 8 and 24 h) in three replicates under excess iron.

Gene	Plant tissue	Time Course								
		2h	2h	2h	8h	8h	8h	24h	24h	24h
	Shoot tissue									
Ferritin-1		0.29	1.0	3.03	0.47	0.79	1.45	2.7	1.41	0.83
IRT-1		0.37	1.47	0.38	0.15	0.44	1.79	1.38	1.0	0.20
	Root tissue									
Ferritin-1		1.81	3.29	3.05	0.22	0.52	1.45	0.64	0.32	0.82
IRT-1		1.70	5.03	4.59	0.30	0.55	1.73	0.73	0.44	1.14

Table 7.5. Significance of differential expression of samples over time (TC) in excess iron in relation to control samples in shoot and root tissue of CDC Redberry genotype.

TC (h)	Gene	Tissue	N	E	SE	95 % CI	P(H1)	Remark
2h	*Ferritin-1*	shoot	3	0.474	0.241 - 0.938	0.159 - 1.264	0.199	NS
8h	*Ferritin-1*	shoot	3	1.056	0.763 - 1.411	0.711 - 1.843	0.724	NS
24h	*Ferritin-1*	shoot	3	1.049	0.589 - 2.150	0.377 - 2.792	0.832	NS
2h	*Ferritin-1*	root	3	2.724	1.866 - 4.644	1.342 - 5.267	0	Up regulated, significant
8h	*Ferritin-1*	root	3	0.554	0.310 - 1.018	0.228 - 1.395	0.28	NS
24h	*Ferritin-1*	root	3	0.558	0.383 - 0.796	0.096		NS
2h	*IRT-1*	shoot	3	0.591	0.223 - 1.443	0.116 - 2.883	0.539	NS
8h	*IRT-1*	shoot	3	0.487	0.218 - 1.634	0.162 - 1.835	0.298	NS
24h	*IRT-1*	shoot	3	0.65	0.283 - 1.280	0.211 - 2.734	0.517	NS
2h	*IRT-1*	root	3	3.563	2.186 - 5.066	1.874 - 5.405	0	Up regulated, significant
8h	*IRT-1*	root	3	0.672	0.386 - 1.101	0.313 - 1.640	0.245	NS
24h	*IRT-1*	root	3	0.715	0.504 - 1.129	0.441 - 1.382	0.192	NS

Here, N= number of biological replications, E= Differentiial expression, SE= standard error, P (H1)= Probability of alternative hypothesis

Expression analysis of Ferritin-1 and *IRT-1 gene*

Using the $2^{-\Delta\Delta CT}$ method (Livak et al. 2001), changes in gene transcripts were calculated for the treated samples (under excess iron condition) compared to the control treatments (iron-deficient condition) (Table 7.4). For *Ferritin-1* and *IRT-1* genes the changes in gene transcript were not significantly different for the shoot tissue (Table 7.5). A 2.72 fold increase in *Ferritin-1* gene transcripts was observed in root tissue after 2 h of iron treatment ($P<0.05$) (Table 7.5). Similarly, a 3.56 fold increase in *IRT-1* gene transcripts was observed ($P<0.05$) (Table 7.5).

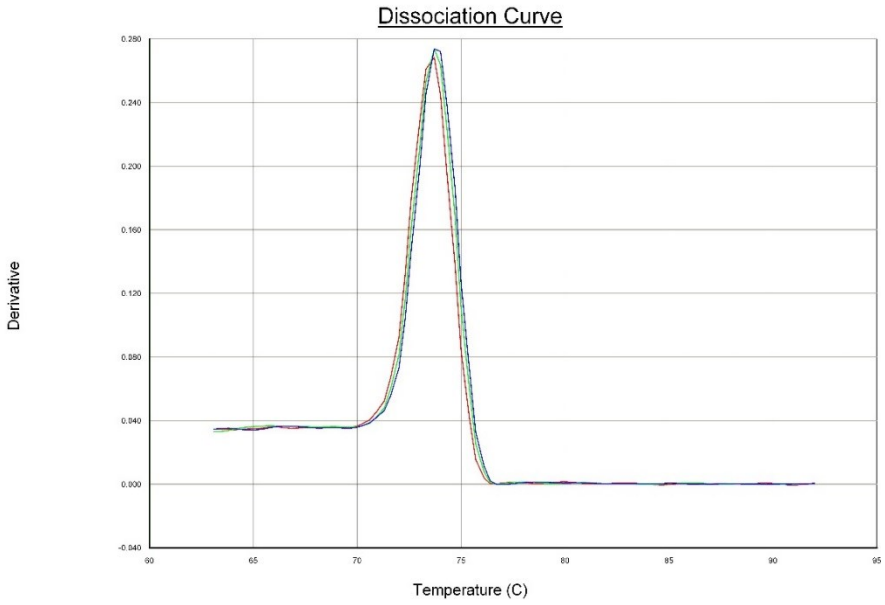

Fig. 7.3 Dissociation curve for *Ferritin-1* primer pairs. Derivative plotted in Y axis is the negative of the rate of change in fluorescence as a fraction of temperature and temperature is plotted on the X axis of the graph.

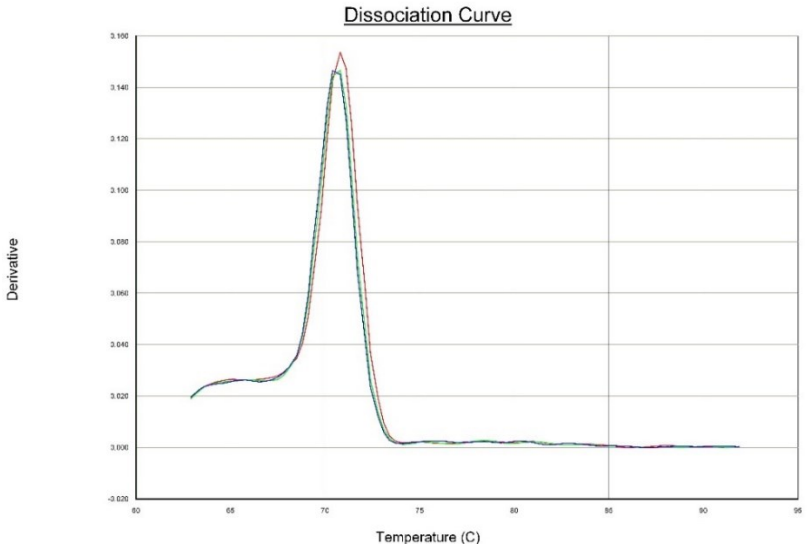

Fig. 7.4 Dissociation curve for *BHLH-1* primer pairs. Derivative plotted in Y axis is the negative of the rate of change in fluorescence as a fraction of temperature and temperature is plotted on the X axis of the graph.

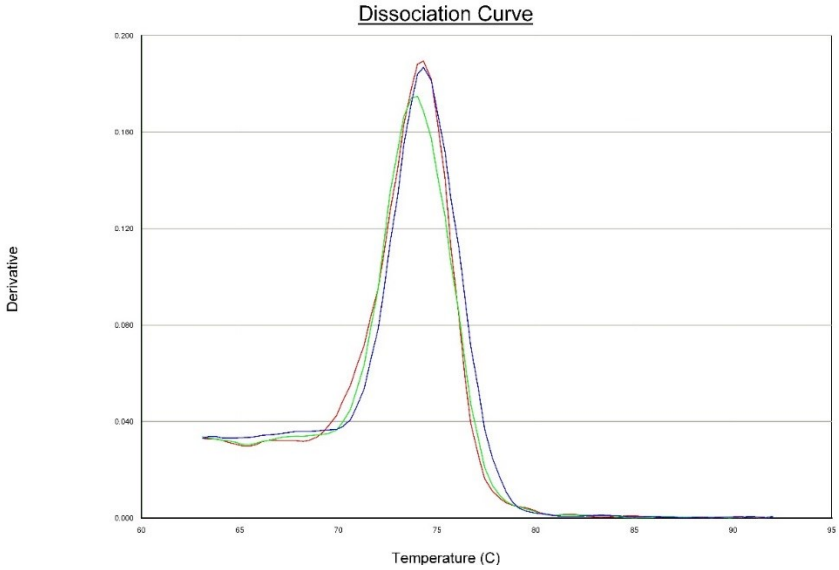

Fig. 7.5 Dissociation curve for *IRT-1* primer pairs. Derivative plotted in Y axis is the negative of the rate of change in fluorescence as a fraction of temperature and temperature is plotted on the X axis of the graph.

Discussion

Iron uptake from the soil and translocation within the plant is a complex physiological process. It involves multiple genes and transcription factors. The magnitude of mRNA transcript

synthesis under excess iron conditions for iron metabolism related genes (*Ferritin-1*, *BHLH-1*, *IRT-1*) in lentil was evaluated in this study. Two genes, *Ferritin-1 and IRT-1,* were quantitatively assayed for differential gene expression while *BHLH-1* primers failed to exhibit amplification efficiency above 90 percent. Ideally gene-specific primers with amplification efficiency >90 percent are considered for qPCR experiments (Udvardi et al. 2008).

Dissociation curve analysis (Figs.7.3, 7.4, 7.5) which is the dsDNA melting curve analysis (Udvardi et al. 2008) added at the end of PCR run showed the specificity for single amplicon amplification and expected melting temperature for the individual primer pairs. All of the three primer pairs exhibited a typical single peak with expected melting temperatures (Fig.7.3, 7.4, 7.5). Gene expression quantification values (C_T values) were normalized using geometric means of C_T values of the two reference genes (*GADPH*, *Actin*) (Vandesompele et al. 2002). *Actin* and *GADPH* were used in studies in lentil, pea and common bean exhibiting stability of expression across tissues and plant parts (Saha and Vandemark 2012, 2013, DeLaat et al. 2014). The objective behind the normalization of qPCR data was to remove the sampling error, which may arise due to RNA quantity and quality differences across samples (Table A4).

In this study we developed gene-specific molecular markers for three genes (*Ferritin-1*, *BHLH-1*, *IRT-1*) in lentil. Primers for *Ferritin-1*and *IRT-1*were used in differential gene expression analysis. Partial genomic DNA sequences of *Ferritin-1* and *BHLH-1* were submitted to the NCBI database. These sequences are available to clone full length genomic sequences of each gene in lentil. The partial genomic DNA sequence *BHLH-1* gene can be further analyzed and used to develop qPCR compatible primers for this gene. It can be hypothesized from the comparative genomic synteny of lentil with *M. truncatula* (Phan et al. 2007) that a ferritin gene family does exist in lentil and other ferritin genes in *M. truncatula* (*ferritin-1* and *ferritin-3*)

could be used to develop molecular markers for the respective ferritin genes in lentil. In addition, once the lentil whole genome sequence is released cloning and characterization of ferritin and other iron metabolism related genes will be easier.

In gene expression analysis under excess iron it was observed that only samples with 2 h excess iron treatments exhibited significant differential gene expression (Table 7.5) for both genes (*Ferritin-1* and *IRT-1*) in root tissues. The absence of such kinetics in gene expression change for samples that were given 8 h or 24 h excess iron treatments across the tissues was observed. The possible reason could be the different iron homeostatis mechanisms in lentil compared to other plant species studied under similar conditions. Development of an assay to find out the reason behind such variation could first start with the standardization of external iron treatments in lentil. In common bean by applying identical excess iron concentration (Lobre´aux et al. 1995) in leaf tissue similar kinetics of differential gene expression of ferritin genes (PvFer1, PvFer2, and PvFer1) were observed (DeLaat et al. 2014). Out of the three genotypes (IAC-Diplomata, Carioca, and BAT 477) used there had been significant genotypic differences of ferritin gene expression for two ferritin genes (PvFer1, PvFer2) (DeLaat et al. 2014). There were no significant differences among the treatments (control with distilled water, osmotic shock causing polyethylene glycol (PEG) treated, excess iron treated, PEG + excess iron treated) for any of the ferritin genes (DeLaat et al. 2014). The interaction between time and treatment was only significant for the PvFer2 and interaction between time and cultivar was significant for PvFer3 ferritin gene (DeLaat et al. 2014). In most of the treatments ferritin genes were up regulated, however, there were treatments where PvFer1 and PvFer3 were down regulated (DeLaat et al. 2014) over time. The above mentioned facts for common bean ferritin genes support the results we obtained in the case of *Ferritin-1* and *IRT-1* genes under identical

conditions. Further, the gene expression levels for iron metabolism related genes were low in lentil as evident by the high C_T values. Number of biological replications may be increased to improve power of the test. The difference between seedling and adult plant physiology should be taken into consideration in future experiments. In summary, gene specific markers were developed for 3 iron metabolism related genes (*Ferritin-1*, *BHLH-1*, *IRT-1*) in lentil using PCR based cloning and significant differential expression was observed for *Ferritin-1* and *IRT-1* genes at the transcriptional level.

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CHAPTER 8. OVERALL CONCLUSIONS AND FUTURE RESEARCH

Micronutrients act as cofactors for enzymes and vitamins act as coenzymes in metabolic reactions. Both these activities are important for normal growth and development of human body. Iron deficiency is prevalent throughout the world. Folate or vitamin B9 deficiency in child bearing women causes neural tube defects in child. Preventing iron and folate deficiency occurrence is a priority research area. Lentil (*Lens culinaris* Medik.) is a food legume consumed heavily in developing countries of Southeast Asia where iron and folate deficiencies are prevalent. Biofortification of lentil for iron and folate concentration will positively impact the nutrition status of the consumers. To initiate any biofortification program phenotyping of the existing germplsm resources is important to identify donor sources. Phenotyping was done to find out the present status of lentil genotypes for folate concentrations. It was observed that lentil genotypes used in this study were rich in bioavailable tetrahydrofolate concentration (255 µg/100 g). Significant genotype x environment effect was observed controlling folate concentration in lentil. Folate concentration is significantly influenced by varying environmental conditions as reported by other workers working on secondary metabolites. In this study only cultivated species were evaluated for folate concentration; inclusion of wild species may exhibit the broader range of diversity for folate. Future experiments involving field samples should take into consideration of the soil fertility status also.

Cultivated (*L. culinaris*) as well as wild species (*L. ervoides*, *L. nigricans*, and *L. lamottei*) were used for mineral analysis. Lentil being a crop with narrow genetic base like any other food legume inclusion of wild species was important. Phenotyping of 26 lentil genotypes were done for iron and other micronutrients (zinc, calcium, magnesium and copper) to know the mineral profile. Significant differences were found between 26 cultivated and wild lentil

genotypes for micronutrient (iron, zinc, calcium, magnesium and copper) concentration. No single genotype had high concentrations of all micronutrients. Genotype with high concentration of any specific micronutrient can be crossed with a genotype with low concentration of that specific micronutrient to develop mapping population. Mapping populations can be used to map the regions in lentil genome controlling micronutrient concentration. Future studies may involve larger set of germplasm or core set of germplasm for phenotyping micronutrients in lentil. It is important to know the proportion of everyday requirement of the body for any micronutrient or vitamin fulfilled by each serving. The data from phenotyping were analyzed to find out the proportion of recommended daily allowance (RDA) 100 g serving size of lentil can provide. It was observed that 100 g serving of lentil can provide considerable fraction of RDA for the micronutrients (Fe, Zn, Cu, and Mg), for example, 100 g serving of lentil could meet 14-51% of RDA for iron. However, 26 lentil genotypes were not a good source of Ca as observed in the study.

Part of the present research concentrated on the genetic aspects: use as well as development of molecular markers to analyze genetic variation in lentil genotypes. Molecular markers were used to know the genetic relatedness of the lentil genotypes. A limited number (13) of simple sequence repeats (SSRs) markers were polymorphic and the cluster analysis grouped 29 cultivated and wild lentils genotypes into 4 clusters broadly based on the genotyping data, which was at par with their pedigree relationships. Number of alleles amplified ranged from 2-4 which indicated the limitation with agarose based allele detection electrophoresis system. More number of SSRs as well as other types of molecular markers, for example, single nucleotide polymorphism or SNP markers could be used to study the genetic diversity in cultivated and wild lentils.

Polymorphic simple sequence repeats markers are limited in lentil. Public databases were used in many crop species like rice, wheat, chickpea, pea, to develop genic simple sequence repeats markers. This approach reduces the cost as development of libraries or clones are not involved. A set of 57 polymorphic expressed sequence tags-simple sequence repeats (EST-SSRs) markers were developed from 9513 EST sequences of lentil available in National Center for Biological Information (NCBI). A diverse set of traits was assigned to these genic markers. The number of alleles amplified ranged from 2-17 for each marker which indicated usefulness of capillary gel electrophoresis system compared to less powerful system like agarose gel electrophoresis for allele detection. These polymorphic primers along with annotation data may help in lentil improvement for yield and nutritional traits. For example, one of the annotated markers, *BHLH-1* used to study for iron uptake in lentil under excess iron. These polymorphic primers could be mapped in lentil genetic map by genotyping a mapping population in lentil.

Micronutrient uptake in plants is a complex physiological process governed by homeostatic mechanisms in the plant. There is existing thin line between the concentration of micronutrients causing deficiency and toxicity in plants. It is therefore important to understand that how plant respond to the external excess supply of micronutrients. In case of iron lentil being a dicot plant uses strategy I where ferric iron is reduced at the rhizosphere and absorbed as ferrous iron by the root. Uptake of ferrous Fe into the root is carried out by the metal transporter *IRT-1* (iron-regulated transporter). The basic helix-loop-helix (*BHLH*) transcription factor family in plants is a ubiquitous regulator and is highly conserved, regulating different types of genes during transcription. The BHLH transcription factor or *FIT* (FER-like Fe deficiency-induced transcription factor) is reported to be responsible for high-level expression of *IRT-1*. Another important gene in iron metabolism is *Ferritin* which codes for iron storage protein ferritin.

Ferritin protein with a cage like structure which carries upto 4300 iron atoms per molecule. In order to understand the gene expressions under external excess iron gene specific markers for three genes (*Ferritin-1*, *BHLH-1*, *IRT-1*) were developed using sequence information in *Medicago truncatula*. *Ferritin-1* and *IRT-1* were differentially expressing under excess iron at 2 hours and not at 8 hours and 24 hours. This transient increase in mRNA of *Ferritin-1* and *IRT-1* and further decrease in gene expression over the time course (8 and 24 hours) further supports the existing strong homeostatic response. It is now important to observe the gene expression at translational level under excess iron. Future study may involve strategies to develop quantitative PCR compatible markers for other iron metabolism genes in lentil such as other members of *Ferritin* gene family and other transporters involved in iron uptake and translocation. More emphasis should be given to create actual soil condition under external iron supply. The difference between adult plant and seedling plant physiology should also be taken into consideration.

Results of these studies contributed to a broad understanding of the genetic variation, environmental influence on and expression of genes related to micronutrient and vitamin concentration and metabolism in lentil. The approach applied for iron and folate concentration can be applied to other micronutrients and vitamins.

APPENDIX A. TABLES

Table A1. Details of 39 molecular markers used for the genetic diversity analysis of 29 *Lens* genotypes (Gupta et al. 2012; Kaur et al. 2011).

Primer name	Type	Forward primer	Reverse primer	Amplicon size
BE325495	EST-SSR	CAGCCACATTTTGC TGTAAAGA	AGTAACCTTTGAC CCCAGCAT	300-330
BE323614	EST-SSR	GCACCAGGAATAAT CCAATAACA	AGCCGTCCAGTAC CTTTGAC	350
TC16680	EST-SSR	TGGAGCCATCAGAA TTCCTC	ATTACGATCCACC AGGCAAC	75
AC123571	SSR	CTGATCCTTTCCAA GAAGCG	CGCTAATTGCTGG CTTCAAA	190
AC139354	SSR	TGAGAGAGAGAGG GCGAGAG	AGGGGCTTTTGCC TATTGTT	275
AC139748	SSR	ATCTGGTAGGAGAT GGTGCG	ATGCAGAGGGGTG ATTCAAG	150
AC143341	SSR	CACGTGGGATGTCA CCACTA	GCCTTGCTGCAGA AGCTATT	400
AC146569	SSR	GACAAACGTTCAAT GCCACA	GGCTCCCTCCACT TGTAATG	270
AC146588a	SSR	GGGTTCTATGCATT CTTCGC	CCTCCCTCCCTCTC TCTCTC	410
AC146588b	SSR	GGGTTCTATGCATT CTTCGC	CCTCCCTCCCTCTC TCTCTC	800
AC146588c	SSR	GGGTTCTATGCATT CTTCGC	CCTCCCTCCCTCTC TCTCTC	850
AC148097a	SSR	TTGGTGCACCGTAT TTTGAG	CCAGGCATCCTTT TCTTTTC	700
AC148097b	SSR	TTGGTGCACCGTAT TTTGAG	CCAGGCATCCTTT TCTTTTC	200-220
AC149127a	SSR	GGCTGATTTGAAAC ATGCCA	GGTGGTTGTGGGA CACTTTT	330
AC149127b	SSR	GGCTGATTTGAAAC ATGCCA	GGTGGTTGTGGGA CACTTTT	100
AC149208	SSR	GTTACACCTAGCCC CATCCA	CACCAGAGTTATG CCAGGGT	175
AC153128	SSR	GTTCCAAAAACGCA CCAAGT	CATGACAGCAGTA CATTGCC	550
AC152551	SSR	TCAGCTTCATCAGC CAAAGA	CCAAACAGGGCCA TAGACTC	220
AC157537	SSR	GCGTGGGATCACGT ACTTC	CTCATCCATTGAT CTTTCCG	500–525
CR538722	SSR	GGGTTTGTTGGTAG TCGGTT	TCGAAAAGATGGG TGGAGTC	950

Table A1. Details of 39 molecular markers used for the genetic diversity analysis of 29 *Lens* genotypes (Gupta et al. 2012; Kaur et al. 2011) (continued).

Primer name	Type	Forward primer	Reverse primer	Amplicon size
AC168149	SSR	GGCTGATTTGAAAC ATGCCA	GGTGGTTGTGGGA CACTTTT	475
PBA_LC_0218	EST-SSR	AGTTCTGCTCCTACT TCAACC	GCAGTTGCTGAAG ATATAGGA	149
PBA_LC_0225	EST-SSR	AACTGTTGCTAAAT CTTGTGG	AGCTTGCAAACTT AAATGACA	136
PBA_LC_0237	EST-SSR	AGGGTTTTGATTTTG ATTGTC	TCTTATCAAGATG AGATGTCTTT	118
PBA_LC_0250	EST-SSR	TGCATTTACCATCAT CTCTAAC	TGATTGATTCGGT ACTTTTTG	149
PBA_LC_0254	EST-SSR	ATGTTAATAAGCAG CAGCAAC	AAGTTGCATGTAA CCACAAAC	127
PBA_LC_0273	EST-SSR	TGAAACCTTTTTGA AGACAAG	TCCATCTTCTAGAT TCTTCCA	147
PBA_LC_0278	EST-SSR	GACGCAGAAGATTA AGGAGAC	ATTCTGACCATAA CCATTCCT	160
PBA_LC_0301	EST-SSR	GTCAAATGAAGTGA ATGATGC	ATTATGGTAACCA CCACCAC	244
PBA_LC_0303	EST-SSR	TAACAGCTGAAATA GGCGTAG	TCACTACTCCAAA CTTCTTCG	165
PBA_LC_0315	EST-SSR	CTCTGAGCATCAAT GAGTTTC	GGCACATTACTGT ATGCATTT	142
PBA_LC_0323	EST-SSR	GAATCAGTGTTCGT GTTCAAT	TTGAAGAAACCTG AAGATCAA	150
PBA_LC_0327	EST-SSR	CCAAGAGCCATCAG AAATAG	AGGACTATCACGA AGAAAACC	147
PBA_LC_0341	EST-SSR	AGATCGAAGACAAA GAGGAAC	ATTCGCTTTTGAA GAAGGAT	149
PBA_LC_0361	EST-SSR	TTAAGAAAGGAATG TCTGCAA	AACTACATGGAAA CCCAAGTT	155
PBA_LC_0368	EST-SSR	ACTACCAAAGAAGC AGAAAGC	CTGAATTGCAAAC TTTCTTTG	142
PBA_LC_0369	EST-SSR	AATGAGAGATATTC TTTGATTGG	GTGATAGGACTAC ATGGCAAA	152
PBA_LC_0373	EST-SSR	ATTTGGGCAACATA TTCAAG	ACTATACTTTCTCC CGTCGTT	164
PBA_LC_0383	EST-SSR	CAGCAACAACTTCC TAACACT	GAGTTAGGGTTTG TTTGGTTT	133

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences.

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0001	TGATGACGATGATCCACAGCA	GCAGTGATTCCCACGAAAGC	60	(GA)46	280
UN0001	TGCGCAAGAACCACAGATCT	TGAAACCACGGTAAAGCCCA	60	(A)20	251
UN0002	ACAAAGAGAGCGAGGAAGCC	GAATTCAGCCACGGTTGCTC	60	(A)19	260
UN0003	TGTGTGTTTGGAGCAATGCT	GATGAGGACCTGGACCTCCT	59	(A)12	212
UN0003	ACCATGTATCACTGTGTGGTT	AGTGAAGGGGTAGGTTGTTGA	58	(T)24	230
UN0004	TTTTGGCCAAAGGTTCAGCG	TGAGCCTTGTAGCCAACCTT	60	(T)13	149
UN0007	TGCCACTTTTTCACACCAGA	ACCTGGAGACAATGAGAGAGAGA	59	(A)19	271
UN0017	TGAGGCTTCTCTCGTTTGCT	TGATGTGTTTCAGTGGTGGT	58	(A)19	275
UN0019	TTGGCACGCAACATCACAAG	GGGTATCACGGAGAAGTTGCA	60	(AG)51	275
UN0020	AAGGAACACACACGCAGAGG	AGCTCGCATTGGAAGAACCA	60	(GA)49	259
UN0023	CCTCCCAAAACCTCACTCCC	TGTTGAGCCATGGTGTTTGTG	60	(TA)36	226
UN0024	CCGCTGTTTTTGCACCAAGT	AGCAGCAATAGGAGTAATAGCCA	60	(A)21	275
UN0026	GCGCATCTCGAACAGCAAAA	GCATCGCCCTCTAGTCCTTC	60	(GAT)6	131
UN0027	ACACGCCCATAGAAGGAACG	TCCACTCTCACCAACGGAGA	60	(AG)38	267
UN0028	TTGGTCGCAGAATCAGAGGC	TGGCCAGATTCCAACAAGGA	60	(A)18	276
UN0029	CAGTTCCGTCCTCCGTTTCA	CGAGTTCAGGTTTGTTGGCG	60	(GA)22	280
UN0031	CGGTGGTGGTCTCTTTGCTT	TACGAGGAGCCTCAGTACCC	60	(T)10	279
UN0032	TGTTGGTGCTGGTAAGATAGGT	CCCTAACCAGCCCAAAGCAT	60	(AT)6	255
UN0033	CCCAAGCCAACCATTTTTGC	GCATCAGGTTTGCCACCAAG	59	(A)10	164
UN0033	GGTGGTGGTGTTTTGATGGC	AGCAATCACCTCACTGTGACT	60	(TTA)8	162

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0036	CAGTCCCCCTTTG CAAATGC	GGGTAGTTCTG CGGAAAGCT	60	(A)21	270
UN0045	TCCTCAACTTTCTC ACGGCA	ACTTTTTCATA CAGGTCAAGTT CGT	59	(A)21ctcg ag(T)23	215
UN0046	TCAACTCGCATCC TCTTCACA	TGATTGGGGGT TTGATGGGG	60	(TTC)6	213
UN0049	AGCTCATAGTGAC CAAAGGATGA	GGTGTAAGTGG GACGCTCAA	60	(A)10	222
UN0059	CAACCTCTGCTGA TCTGGGG	AGCTCATAGTG ACCAAAGGATG A	60	(T)20	208
UN0066	GCAAATGCTCAAC CTCTGCA	TCAGCGATCCC AATGACACC	60	(A)19	271
UN0068	ACGTCGTACCCCT CCAATTT	TTCCTGCGAGC GGAGATAAC	59	(A)18	114
UN0069	TGACCAAGGTGGA ACCATCG	CCCTCGTCTTA CGTCGATCG	60	(A)12	248
UN0073	TGATCACCGTGGT TCAGCAA	AGCTCATAGTG ACCAAAGGATG A	60	(T)27	199
UN0075	AAGAGTTGCAGAG AAGCGGC	TGAACCGGAAC AAAGGGAGG	60	(T)10	251
UN0076	GCCAAGGAAGAA GGAGTCCC	ACCATGGATTT GTTGGAGAGGA	60	(A)16	238
UN0079	AACCACAGTCCTC TGAAGGC	TGCATGTGTAC GGTTAGTGCT	60	(T)11	196
UN0079	TCGGGTGAGACCA TTGTTCG	CAGACACCACT TGTTGCTGC	60	(GGC)5	270
UN0080	AGCTCATAGTGAC CAAAGGATGA	CATGGATCTTG GGGAAGCCA	59	(A)19	179
UN0085	AGCTCATAGTGAC CAAAGGATGA	CAGTAGCTGCT GCACTTCCT	60	(A)19	238
UN0087	CCACCACGAAGAT TAGTGTCGA	GCGAGTTGCTA AACAGGCAC	60	(AG)16gg g(GA)30	279
UN0088	AGGCGTATTAGAG GCGAACG	GGCCAAGAATG AGCATTGCT	60	(A)19	208
UN0089	ATGGGAAGTGCCG TTGGATT	CCACAGTTGTT GACGCACAG	60	(A)25ctcg agactagt(T C)21	239

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0090	TGCATGTGTACGGTTAGTGCT	TGATGGGTTTTGTTTGGTGACA	59	(A)18ctcgag(T)18	216
UN0092	CAAGTTGCTGGTCGAAGGGA	AAGCACAGAGGGGTGGAGAA	61	(A)18	270
UN0097	AGACGTGTATCACCATCTCCA	CAGGAGGAGTTCTAGCTGCG	59	(A)18	257
UN0099	TACTCATCGCCGTTGGTGTT	TCCTTAGTTTCAAAACAGCTTTCA	58	(T)20	267
UN0106	CAGGAGCAACTTCCAGTGCT	TCTCCCCCTTCCCCTTTTCT	60	(T)18	279
UN0106	AGAAAAGGGGAAGGGGGAGA	CTTCCTCCCGATTCTCACCG	60	(ATA)6	173
UN0110	AAGCTGATGCTGACATGCCT	CCATAAAAGTATGCCCAACTTGCA	60	(T)11	219
UN0118	TCGGTTTGATGGGTGGTTGT	AGGGTGGATTGTGGCAGAAG	60	(A)26	263
UN0119	ACATTTTGGTTGAAGTCTGCCT	AGCTGCCTTGCCTCATTTCT	59	(A)21	265
UN0123	ACCGTCTGATTGAGCACAGT	TCCAAAGCCATCCAGTTCCC	60	(CT)53	247
UN0125	CAGGGAGATGCTGACAGTGG	CTACTTCCGCACCCACAGTT	60	(A)16	257
UN0126	GCAGCACTCAATTCACCAGC	TGAAAGGAAAGGTTTTAGCTGAGT	59	(CT)9c(CT)13	274
UN0131	GGAAGTGCTTGTGCTTGGTT	AGCAGTTTCTCCAAGCGACA	60	(A)18	255
UN0133	TGATGCCTATGCTTGCGAAGA	CAACCTCGACAGTGGCCATA	60	(A)12	205
UN0135	AAGGGAATGCTGATCGGCTT	GCTCTAGCATTTTGCATGTGACT	60	(T)12	266
UN0136	AGCTCCTGCTCAGCAAGAAG	GAGACGCTGCACCATTTTCG	60	(A)30	254
UN0146	TGACACCAAGGCCACTGAAG	AGTTTGGATGCGCCCCATAA	60	(GAT)7	258
UN0155	CTTGTTGGCCGTTTTGGGAG	CTCCTCCAGTTGCAGCAGAA	60	(TGG)5	227

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0167	CACGTTACGAAGATGGTCGG	AGAAGCTGCCATTTTCCGGA	59	(A)22	245
UN0201	TCGCATTTTTGCTCAGTTCCT	GAAAGATGAGGGCTGGTGGA	59	(T)18	279
UN0222	ACCTCCTACCCCTTCTTCCTC	CAAAACCTGAGGACCACCGA	60	(TCA)5	145
UN0225	ACATGTTGCAATGCTTTTAGCCT	TTCTTGCTTGGCGTTGAAGC	60	(A)29	228
UN0230	AGAGGGCTCCAACTCTGTGA	ACGGGCCGAATAATCATGCA	60	(T)10	159
UN0235	GCCAAGGCCGACAAATAAGG	TGCAAGGGTATTTCTTTTTGTAATTGC	60	(A)13ctcgag(T)28	278
UN0244	ACGTTGAAACATTGGATGTGCT	GGAAATGTGATCAATGGTGGGG	59	(AAC)5	265
UN0250	AGTGTGTGAAGTGTGTGAGTGT	AGTTCCACGGATGAACGCTT	60	(T)10	206
UN0252	GGCTCAGCCTCAAGCAAAAT	ACTTTGGCTTGCCTCTACCG	60	(A)22	230
UN0281	TGTCTGGCTTGAGCAGAAGA	TGTTGCCATAGCTTGCCTCA	59	(A)22	186
UN0292	TGGGAGATGTCTGTTGGTGT	CTGGGTCATCAGCTAAGCCC	59	(A)23	255
UN0314	GCGAAGGAGTCATTTGTTCCA	CGACGAGCCATGGATGAAGT	60	(T)10atgaaatatattttggaattttaatg(A)19	276
UN0322	ATGGCGTGAGGAAAACCCTT	TGGAAAAGAACTGAGAGCCACA	60	(GTT)9	256
UN0325	AGAGACAACCTTTGTTTTGGAGT	TAAACCGGGAGCGTTTGTGA	59	(A)33	227
UN0326	TGCGGATTGAGAAGGTTGTGA	AGCCAAGCAAGCAACCCATA	60	(T)11	277
UN0334			0	(TTA)5	
UN0350	ACCAAGGGACTGAATGCGAT	GGAATGTCGAAACCTGGCCT	60	(T)10	103
UN0353	CGTGGAGGTGGTGGATATGG	CTCCCTCCAGTTTCCACCAC	60	(GGA)5	198
UN0383	CCGTTTGATCTTCTAAGCCCCT	AGGGTCGGCACATTGTTGAA	60	(T)10	199

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0390	TTCAATATCTCTG CCGGCCC	ATCTTTGCCAC TGCCTCCTC	60	(GAA)5	250
UN0400	TGTCGGTTCGCCT TCTTGTT	CCAGCTCCGGT GATGGAAAT	60	(T)11	190
UN0426	GCTTCACCTGACT CTCCACC	GAAGATCGATC CCGTCCGTC	60	(TAT)5	278
UN0428	CCTCGGTTTGCCTT TGCATT	CGTCGAAATCC AGGTTCCCA	60	(A)28	255
UN0470	GGTGGTCCCATCC ATAGCAC	GCACGTTTGCA AGGAAAACA	59	(T)11	277
UN0476	CAGATGTGTCGTG GCCTGAT	TACCACCGACC CGTGTATCT	60	(A)18	227
UN0487	GGGTTTGGCTCGA TCACAGA	CCGCACAATCC AATTTGAGCA	60	(T)10	214
UN0500	TGCGACTTCTCAA GTTGAATGG	TCAAGATCGTT TGCTCATCTGG A	60	(T)18	210
UN0502	CCGTGTTTCGTTTC CCATTGT	ACTACCACCAC CTGTTCCCT	60	(A)16	180
UN0518	ACCGCATGACTTC GAGGAAG	GCCAAGGTTTG CCTAGAAGC	60	(A)18	279
UN0521	GTTTGGCTGCAAC GTTGAGT	AGAGTTCACAA GCTTCACACT	59	(T)13	263
UN0536	ATAGGCCTGCTTG GACCCTA	ACAAAGGCAAT TTCCAAACGT	59	(TA)6	103
UN0538	GCAAAGAGCTCGT GTGTGTT	AGCAGTTAGAT CACAGCTACCA	59	(T)12	128
UN0549	TGCATTTCATGGT TCCCATCT	TGGCGCGCAAT AGAATCTTG	59	(A)19	240
UN0561	GGATGGCACACTA GGCCATT	TGTCCACTCAA CCCCACAAG	60	(A)10	214
UN0566	CTAGCTTCCCACG AAGAGCC	AGCATGCAATC TTCACCCCA	60	(T)19	135
UN0575	CGCTCAATCTCCT TCCCCTG	CCTCCTCCGCG TTCTACAAA	60	(T)12	228
UN0585	AGCTTGATGTATT GTAGCTTCAAAGT	AACACCGATTT TCCCTCGCT	60	(A)15	272
UN0591	GCATCCCCCAACT ACGATGT	TCTGTTGGTAC TTCGGTGGC	60	(A)18	203
UN0596	CGAGGGTTTCGTT TTCTCCC	TGGTTCTTCAT CGCTGCCTT	59	(GAA)5	213

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0607	CGCGCTCTACCAA CAGCATA	CTGCAAGGAGT AGTCGCCTC	60	(CT)19	223
UN0642	TGGAAGTTGAAGC GACGGTT	GAACCCGGGG ACATGTATGT	60	(A)10	222
UN0643	TGAGCAGCATCTC CGATACA	ACCATGCGCAA GGGGATAAA	59	(TA)6	247
UN0660	GGTCGAGATCTCA TGCTGCT	TGAAGAATACG GCGGCGATC	60	(A)18	275
UN0676	TGACCCATTGTTC AAGGAGGA	ATGAGGATATT GCCCTCGGC	59	(A)27	198
UN0678	CAAGCCCTTCTAC GCCTCTT	CCTCCAATCAA GATTCAGCTGC	60	(A)10	263
UN0708	ACTGAGGGGAAA AGGAGGGT	ACAACCTTAAC CCCGGTCAG	60	(GTG)5	143
UN0737	AGGACAAGTGTTG GTGTGGG	CATGAGGGACA GCACCATGT	60	(TAT)8	278
UN0745	AGTCGCATTTCCC GTCTGTT	ACAAACAACTC ATGATGTGCCC	60	(A)12	268
UN0748	CATTGCTGCGTGG TTCAACA	TCAAATATTCA GTGTCATGTTC TACTT	59	(A)10	210
UN0755	CATGCACACCAAA TCCACCA	TATCGGTGGCA CGACAACAA	59	(ACC)5	131
UN0771	GCGCATGCTTATG ACCCATG	ACACAGCTTCG CATCACACA	60	(T)11	220
UN0788	CGACCAGGAAACG GCAAAGA	AGCCTCGATCC CCTTCTCTT	60	(AGA)5	241
UN0792	CTTGTTTCTCCGCG CTTTCC	TCCACCGTTTG GACGAATGT	60	(TAG)5	238
UN0832	TTCGCCTTCCCAT GTCTTGA	ACAGCATAGCA TAGCTACAACT	58	(A)18ctcg ag(T)22	209
UN0849	TTTCTCATCACCA CCACCCA	GAGGGCGATTC TGCTGCTAT	59	(CTC)5	192
UN0853	TGGTTCAGGGTGC GAAATCA	GGCTCTACCCT GTGAAGCAT	60	(A)27	277
UN0854	AGCTGTAAGGCAC TGTGTGT	AGTGCTGGTGC TTCTCCAAG	60	(A)27	236
UN0855	CCGATCGTTTTTG CACAACCA	GGAGGTGGTTA TGGTGGTGG	60	(CCA)5	259
UN0856	TGCGAGTTTGATA AACCGTGT	CCAGCCGCAAC AACAACTAC	59	(TGG)5	166

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0859	TGCCTTGGTCAGTGTCAACT	TCTGATATCCAGCTGACTGAGC	59	(T)21	276
UN0860	TCTCCCCGAGTCCATTTCCT	GATGAGGACCTGGACCTCCT	60	(A)19	193
UN0861	ACAACACCATGATGAGCCTTG	TGTGTCATCCATGGACCACA	59	(GAA)10	256
UN0863	GATCGTGAGAGTTGAGGGCG	CTGAGGCGTAAGTAGCGGAG	60	(GAG)5	246
UN0867	CCGCATTATCAACCCCGAGA	TAGCGGAGGATTGGGAGAGT	60	(GAA)5	255
UN0874	TTCGGACTTTCTCGGAAGGT	AGGCCTGGCATTTTGCTTTT	59	(A)20	213
UN0881	TGGTGGTCATCATGGTGGTG	GTAACCACGCTTGAGATCCCT	60	(A)19	274
UN0881	TCAATTTGCTGCCGAGGGAT	CGGGAAGGGTGAGCAATGTT	60	(A)19	249
UN0884	TCAGCATGTGTTTTTGGGCA	CCCTCCGAGCAAACTTCCTT	59	(A)18	249
UN0894	TACACAGAGCACGCAAGGAG	ACCTAACCTTCACCACAAACCT	60	(GAA)5	196
UN0897	TGGAGTCTGAAGGTGGTGAGA	TGCGGGTGCAGTTTGAGTAA	60	(TAG)5	147
UN0898	CAATGGCTCCAACAAAGGCC	CCCATAGCCTTGCTGGAGAC	60	(AAG)5	223
UN0912	CCGCCTGCCTAACCATCAAA	ACCGCAACCTTCTTCTGCTT	60	(A)21	239
UN0916	AGACTTGTTGTGTTCATGCATGT	GGCATGGCAGTAAGGAGGTT	60	(A)14	197
UN0931	AGGGACAAGGAAAATGCCCT	AGCCCTGTACATCACCCAAA	59	(A)16	261
UN0933	TAACACGGCCGGACATGAAA	TACTGCCTGATCGTGCAGTG	60	(T)28	218
UN0945	GTGTTTGGACTTTACGGCGT	TCACATGACCCATCCTCATCC	59	(CAATG G)5	261
UN0948	CCAATCATGGCTTCTGCTGA	ACCTAACAAGTTTCACCGTCT	58	(AG)8	153
UN0949	AGTCACTGTGGTCTGATGAACT	TGAGAGGCCAGTGCTTAAGC	60	(A)12	181
UN0953	ACCTCGCAGCCATGAGATTC	GCTCTCGCGAATCTTTGCAG	60	(A)11	194

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN0956	CCGCAGTCATCCTCTTCACC	TGCATGTGTACGGTTAGTGCT	60	(A)20ctcgag(T)19	259
UN0960	ATCGTCTTCTTTCTCGCGCA	GCGATTGAGAGAGGGAGACG	60	(TC)26	147
UN0962	ACAACACCATGATGAGCCTTG	TGTGTCATCCATGGACCACA	59	(GAA)6	237
UN0967	TGCTGGTATAGTGACCCAACA	CCCACAAGTTCCAAATCCCCT	59	(A)19	138
UN0967	GTGAGCAAGGAATAAAACGAGCT	AACATTTGCTTGCATATCAGAGT	58	(A)23ctcgagactagttc(GA)64	275
UN0975	CCAGGGTCAACCAGGAGAAC	TGCATGTGTACGGTTAGTGCT	60	(T)20	202
UN0982	TGATGGTGCGGTTTCAAGGT	CCTACTCCCCCATCCAGGTT	60	(A)18	270
UN0990	TCTTTACGGGTTTGGCGGTT	TCCCTGCCTCTCCACAACTA	60	(A)26	272
UN1001	TGTTGACCACCGTTGTGACA	TACTGCCTGATCGTGCAGTG	60	(A)19ctcgag(T)24	275
UN1003	TACTGCCTGATCGTGCAGTG	CCCATTTGCGAGACTCACCT	60	(A)20	252
UN1006	GTGAGCAAGGAATAAAACGAGCT	CCTAGTGTTGCTGGTGCTGA	60	(A)18	234
UN1009	TGTTCTTCGGCATGGCTGAT	TGCGCAAGAACCACAGATCT	60	(T)18	226
UN1010	TGCCGTGGATTCCGTCATAG	ACATGGCCAAAACCACTTGG	60	(A)18	257
UN1011	TACTGCCTGATCGTGCAGTG	TGGTGGTTTGTTTTCGCACC	60	(A)20ctcgag(T)18	160
UN1012	TACTGCCTGATCGTGCAGTG	GCTTGTTCTGCTTCCTTGGC	60	(A)20	172
UN1014	GCAGCGCAAAAAGTTTAACTCG	TGCCTCTGCCACCATACTTG	60	(A)19	235
UN1014	AGCTACCTGGCTACCCATTT	GGATTTGCGAGCGGTTTGTT	59	(A)19	195
UN1017	TGCATGTGTACGGTTAGTGCT	TCCTCCACAATGGGTTGCTC	60	(A)18	206
UN1018	TGCGCAAGAACCACAGATCT	GCGTGAAGGGTAACAACTGC	60	(A)21	262
UN1019	TTACAGTCTGCTGCTCCTGC	CGTGTGGTCCTATCCTCTTGT	60	(T)21	267

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN1020	GCCCTTCTTAGGAAAGGCGT	TTACAGTCTGCTGCTCCTGC	60	(A)28	267
UN1020	TGCATGTGTACGGTTAGTGCT	CCGGCCTTCTTGGTTCTCTT	60	(A)10	151
UN1021	TGTATGGCCAGTACACGGATG	CGTGTGGTCCTATCCTCTTGT	60	(A)18	197
UN1022	AGTCCCTTTGGCTCTGTTTGT	GCCAACAAATATGCCGCGAT	60	(A)24	250
UN1023	GCGTGGATCCGATCTCTGTT	ATGACCACCATGACCGTGTC	60	(A)19	261
UN1023	TGCATGTATATGTGGTGACGAGT	ACTGCTGACCTTCACTGCAT	60	(A)17	248
UN1025	TGCATGTGTACGGTTAGTGCT	TGTTGCAGTGATTCCCACGA	60	(A)18	161
UN1028	CGTTGATGACGCAGCAGATG	TCCATTACAAGATACTCTCCATGCA	60	(TTTTA)6	249
UN1030	TGCAGCAAGAATGAACTGATTTCT	GGAAAGGGAACGGGAATGGT	60	(A)13	277
UN1038	AGAAGCTCTATTCAGTTGTCCAA	GAGCGAGGAGGAACCGAAG	59	(A)19ctcgag(T)18	280
UN1051	TCACTTGGATTTACAAACACGCA	CCAGCATGACCCTGATGTGT	60	(A)10	228
UN1054	TTGGTTATGGTGCGTCTCCC	TCACCGTTCAGGCACATGAA	60	(T)10	275
UN1064	TTTGGAAACCCTCCTCTGCC	GAGGTGGCATCAGTCCAACA	60	(ACC)5	209
UN1065	GTGCTTATGCTTTTCTGCCAGA	ATCCTCCTGTGAAATGCCCG	60	(A)19	166
UN1066	GTAAAGGAAGTGGGGCAGGG	TCAGCTGGCTGTACAAAGGA	60	(A)10	243
UN1080	AGGGTGGCCTTTGCTTTTTC	CGTTATACACCTGCACCCGT	60	(A)19	242
UN1085	GACACCGCCCAACTCGAATA	TGAAAGCCAAAGGTGGAATCA	59	(T)11	253
UN1097	TCACTGGCGTCGTACCAAG	AACAGAACGGGTGCATCTCG	60	(A)19	280

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN1107	TCATCTTTCTCAAC TCCATAATCATC	TCTCTCCCTGG GCTTGTATGA	59	(CAA)5aa tgttgtctcta ctggccttcg tttatcattcg atgatcaaca acag(CAA)5agattaca gttacaacttc atcaatttcaa tct(CAA)1 1	278
UN1128	CACCAACAACAAC AGCAGCA	CCAACTCCTCT TCCGGCATT	60	(A)10	210
UN1146	ACATTCAAAATCC ACGACGTCG	GGGACCCACTT ATATGGCCG	60	(ACA)5	144
UN1160	GTCGGTGAACCAC AATGGGA	AGCTGCGAACA AGGTGAGAA	60	(T)11	277
UN1242	TTACGCACTCAAG AGGCAGG	AATCTTTCACA AACGCCGCG	60	(A)30	260
UN1250	CGCGTCTCTTCAC TTCCCTT	ATGCTTCACGA AACGAACGC	60	(CTT)6	276
UN1251	TGAGCATTTTGTG GAGTCAGT	TGCGGCTTAGG CTTCAAAGA	59	(A)10	213
UN1258	TTGGCTCAGACTG CACTTCT	TCTGCAGCTTT CCCACCTTT	60	(A)29	237
UN1261	GGAAAAGCTGTTG ATTTTGGCG	TAACGCCGATT CCGATGGAG	60	(TC)10ta(TC)6	169
UN1262	CGGAAACCGCTCC ATGTTTG	TTTGAAGGGCC TCATCCGAC	60	(GGC)5	223
UN1279	CAGATCTTGTTTG GCGCAGG	CACGCAGAGTA AAATCACGTGA	60	(T)11	249
UN1284	ACCTCCTGCAGCT ACTGTTG	TGGTCCAACCT ACCAACTCA	59	(GAA)6	276
UN1296	ACGGAACACATGT GGCTCAT	ACCGTTGCCTG TAAGTGGAA	60	(T)10	267
UN1298	CCTGGATGGATGC TTTGACCT	GCCCATGTCTT TGGCTAAAGT	59	(A)10	191
UN1299	AGTGCGAAAGAGT ACCGTGT	CGTTAACAGCA AGCGCAGAA	60	(T)10	267
UN1300	ACCGGATTCGAAC ACCTGAC	GACCATTGCGT TCCCAATCG	60	(CTC)5	268

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN1304	TCAGGGCTTGCAC TTGATGA	TTTGACCCGTC GTTTTCCCT	60	(A)25	217
UN1349	CTAGCATGGTTGG GACACTGT	TTCTGGCCAAG TGATCGCAT	60	(A)31	269
UN1437	CCACCTGCTGGTT ATCCTGG	GACCATACCCA CCATGTGCA	60	(GCT)5	172
UN1438	CGAATTATCGGAT GTGGCGC	AGTGAATAACT CAATTCAACAA GTTCA	59	(A)11	277
UN1443	CATCAACAACCGT AACCGCC	TGGTCCATTAG GAGAGGCGA	60	(TCT)5	175
UN1449	GATCCGTTTTCTCC ATGCCG	TCTCCATGCTT CTTGTTGCT	58	(CAA)5cc tt(AAC)6	235
UN1464	ACATGGTCAAACA CTCGAGTTG	TGGAGGAACCC TAGATAGGAGT	59	(TAT)6	242
UN1469	AGGAGCAGCATAA TACTCTTGAT	ACAAAGAGAG CGAGGAAGCC	59	(T)10	207
UN1470	TGATCCATGGCAG CTTCCTG	TTCCAGTAACC ACTTCCGGC	60	(T)10	220
UN1471	ACTCAGGTTGTGG CTGGAAC	GGTAACCCTCG TGCCGAATT	60	(AAT)6	262
UN1474	GGTACTCACCGTT AAGTGGTT	TCTCCCATTGC TTCCTCTTGT	58	(A)25	277
UN1541	TGACTGTGTGCTT TTACTTCTGA	TGTTTGCTGCC ACACAAAAGT	59	(A)19ctcg agacgaact agtctcgag(T)19	176
UN1548	GGAACGCTTTCTC GCTGGTA	GTCTGTTCACC ACCAGCTGA	60	(A)18	205
UN1577	AGGGCTTCTCCTT ATTGTGAACA	AAGCACCACCA ACACTTCCA	60	(ATG)5	247
UN1583	CTTCCCGATCGTC GTATCGT	TCAATTTTCTG CATCATGAACC T	58	(TAT)5	262
UN1603	ACATCATGCTTCC ACTCCCG	AGCGAATGGTG GTGGAAGAG	60	(AAC)5	151
UN1616	ACAAACTCCCTCA CCACACTC	GGAACGAGATC AGCAGCCAT	60	(A)10	157
UN1617	AACTTGCCAGACT TCGCAGT	GGTGGCAGTGA TGGAATGGA	60	(A)13	261

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN1622	TGGTTAAAAGGTG ATTGTTTGCCT	ATGGCCAAGTT TCAGCTGCT	60	(T)10	178
UN1645	ATGGACTTGCCGA GAAGTGG	GCCAGAGAGTT CCATGGCAT	60	(GAT)5	215
UN1652	ACATCCCCCAACA GATAGTAGT	ACGATCCAGTT TGCAAAGGGA	59	(A)18ctcg ag(T)19	215
UN1678	ATCGGTGTTGCAG TTCCTGT	GCAGTACAGAG AAATTGATTAT TACCA	59	(AT)7	239
UN1708	TTCCAGCCAAGGT CTTAGGA	AACACAGCACC TTTGTTGCC	59	(A)28	271
UN1717	TTGTCTTTGTCAGC AATACAACTT	CCTACCCGACA TGGATGCAT	59	(A)10	228
UN1724	TGAGGCGGCCACA TACAATT	TTGTGGTCACG ACTCACGAG	60	(T)11	145
UN1753	TACATCTACCGCC ACCCAGA	TTCAGCGAGGG TACGTTTCC	60	(CAA)5	202
UN1761	TGGAGTCTGAAGG TGGTGAGA	TGCGGGTGCAG TTTGAGTAA	60	(TAG)5	147
UN1768	AGGAAACCCAAA ATGCCCTT	TTTGCCGACGA GAGAGTGAG	59	(A)18	244
UN1828	GGCGACGATGATG GTTTCAT	CCGAAAAGGGT AAAACGGCA	59	(A)10	246
UN1839	TGGTGTTTGCATT GTGGAGT	GGCGGGCAAG GTACAATACA	59	(A)13	267
UN1849	GTCTGGTGCCGAG TTCAGAA	AAACCCCTTGT ATCCGCCTG	60	(TAA)7	242
UN1866	AAAAGTCCGGCGA AGAAGGT	AGGAACGGTGT CGAGTACAA	59	(TTA)6	250
UN1867	CCCTTCTTTACCA AACACCAACC	ACCATTCATCA CTGCACCTTCT	60	(T)11	218
UN1875	CGCACTGATCGTA GCAAAGC	ACGGTTTATCC AAATTGCATAA CA	59	(T)10	238
UN1892	TCTGGTGCAAGTC TGGTGAC	AGCTAAGCCAT CCACTTGCA	60	(TTG)9	180
UN1922	GTGTTTGGACTTT ACGGCGT	TCACATGACCC ATCCTCATCC	59	(CAATG G)5	261
UN1934	AATGGAGTCTGAA GGTGGCG	AATCTCAGGAG GGTTTGCGG	60	(TAG)6	167

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN1951	CCGGACCTGGAGC TGATTTT	GCGTACTAAAT CCCACCACCA	60	(T)14	241
UN1952	AGGACAAGTGTTG GTGTGGG	CAGTTCTAAAT CACTGCATCGC A	60	(TAT)8	243
UN1954	TTGTGCAGGGTGG TTTGGAT	AGCTGTTGGTT CAACTGTTACA	59	(T)11	278
UN2000	GCCAAGGTCACAC ACTCACT	CCGCGGTGGAG TAATTCTGA	60	(CAC)6	200
UN2000	TCTTCGTCGTCTTC AGCACC	CCTCCTCGGTG ATGATCTCC	60	(ACA)5	119
UN2014	ACCATGGTCGAAT CTTCTCCA	ACGGTTTTCTT AAGAGAATCG AAACA	59	(A)10	157
UN2098	TACCCTCCGTCCC TCTTCAG	TCTGGTTTAGC CGCACATGT	60	(TC)6	221
UN2106	AGGAGGTGAAGCT CTGAATGA	GCTATGTGTAT GTGGTTTGGCA	59	(T)12	232
UN2107	TGCAAGCCTTTCT AGGAAGGG	GGACATCATCA CCACCAGGG	60	(A)17	244
UN2116	GGCATGTTCAAGT TCAAGGGC	TGTTGCTGCTG TTGTTGCTG	60	(CAA)5	202
UN2116	GTTATGTGTGGCT GGGGTGA	TGTGTATGCTT TCCAGTCAAAC A	59	(A)11	193
UN2132	GTTGCAGTTTTGA GGGCGAG	ATGTTGCTCAG CCCTTGAGG	60	(TTC)5	197
UN2139	TGGATCACTTGTT AACCATCTATAAG A	GCCAAAATAGT TCATTGAAAAC GCA	59	(T)11aaaat tcaaaaaatg atgtgaaata aacca(AT) 8	197
UN2139	GCGTTTTCAATGA ACTATTTTGGCA	TGGCGTTTTCA ATGTTTGTGGT	60	(T)11	236
UN2154	GTTGCGCCAAAAA TTTCCGC	AGCACCAAAGC CCTAAGGTT	60	(TC)6	228
UN2157	CACAAAGCAAAG AGCCACGT	CCCGTGATCAA GGCCGATAA	60	(A)10	219
UN2178	CGTCGTGCAATCA GAGACCT	GTGCATCCTCA TCCCAGTCC	60	(A)10	272

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN2184	TGAGGAAGAAGATGCTGCACA	TTTTCCCAGGGTGAAGGTCG	60	(A)18	221
UN2189	AATCTCAAAAAGATCAAAGAAGAGGA	ACACCCAAAAGAGCAGTTCCA	59	(GAA)7	254
UN2195	TCTAACACATTTACAAAGACTCCAAA	TCCACAAGGAGCACTAACCC	58	(A)10	242
UN2207	TGGGTTTTGATTTGGTGCGG	TTGGAGTCGAGAGCAAGAGC	60	(GCGT)5atgtattcgtataatcggtgac(AGA)9	273
UN2225	CTTTGTTGTGCCTCAGTTGGT	ATGCGAGTGCTCCTTCTTGG	60	(A)18	201
UN2252	CCCGGCAAATTCCTCCAGAT	TGTTGCCTCGATCAAGACCC	60	(GTT)5	257
UN2278	CTCCCTCAAACACCTTCATTGC	AGTCGGTGCGAAATTCGAGA	60	(CAA)6	227
UN2295	GCTTTTGGTTGATTATGTTTTTGAAGT	TGATTGGGATGATACAAAGTGGA	58	(A)10	113
UN2295	TCCCAAATCATATTCGTTTGGCC	ACTGATACCCTGCAAAGTGC	59	(A)10	278
UN2298	TTCGCCTTCCCATGTCTTGA	AAGGCCTGGCATTTTGCTTT	59	(A)19	167
UN2333	TTGTTGAGGATCCGGGAAGG	GTCAGCAAGCAAACCAACTGA	59	(T)14	199
UN2339	AGGTCTTCGCGAACTCACTG	TGTCATGCAAGGTCGTGTCA	60	(GT)8	271
UN2374	ACACACAATATACCGCCCGA	ATTGATTCCTTTGGGGCCGT	60	(A)20	207
UN2389	AACGGCAGATCTTGATCCGG	ACACAGTATCAAGGTGAACATGA	59	(T)11	263
UN2393	TTCCTTGTTTGAGTGGCCCA	AGCCTCTTGGGTCATCTTCT	59	(A)25	269
UN2424	TGCACCATCTTTTGCCTACA	AGAGTGAGTTGAGTCTTGTTATGCT	59	(A)19ctcgag(T)22	171
UN2434	TCCCCTTCTATAAAATCCCTGTTT	AACATGTCACGGATCCGCTT	59	(TAT)7	229

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN2469	TCCCCCTTTAGTG CAGTTTTGT	GTGCCAGAGAC GCTTCTCAT	60	(A)18	142
UN2474	AATGGGCAACAGG TCCAACT	CACTGCATTGT CTCCGACCT	60	(A)34	228
UN2496	CGCTCACGTCTCC TTTTCCT	GGTGGCGGTGG TGGAATAAT	60	(TA)6	127
UN2516	GCAGATGCAAAGG CTATGGC	ACTGTCCAAAG TCCAAGCAA	59	(A)10	208
UN2522	AAGCCAAAGAGA CATCGCCA	ACAGCACATAA CAAAATGCAAC G	60	(T)10	268
UN2538	TGGTGTCAAGGTG AAACCCA	CGAGGAGGAG TGATTCGACG	60	(A)19	200
UN2548	TCTTCTCGCTCGTT TTCGCT	TCATCATCCTT AATCACTTGGG GA	60	(AT)29	280
UN2576	ACATGCGGTTTCA TTTGGCC	TTACGATGATC GAAGGGCCG	60	(T)10	234
UN2594	TTCTTCTTCTCAAT TCAGATCAACTT	GTACCTAAGCT GCTGGGGTC	58	(A)11catata tagcatctatt aaaacatac atgatggaca agcaatttctc aac(A)12	201
UN2605	TCGCTCTCTCTCTC ACCGTT	GGAAGAGAGA TGCGCGAAGA	60	(CT)8	124
UN2614	CCACAACAAACAG CTGCTCC	AGATGTTACAT TGGGGCAGCA	60	(T)11	133
UN2615	TTGGACATTTTTG AATGATTTTCAGT	CACTCATGCTT TCCTTTGAAAA TAAA	57	(AT)6aa(AT)22	280
UN2646	GGAAACGGCGCA AACTTTCT	GTTTGTCCAAA CGCCACTGA	60	(CAA)6	278
UN2649	CCCTCATAGGCCA AAAGGGT	ACAGCAACCTC AGCATCACA	60	(T)10	251
UN2659	ACACGTGTTGCCA TCTCCTT	GCTGACCAAAA TCAAGGGCG	60	(CTT)5	124
UN2661	CTAATGTTGGCAG GTGCGC	CGCTATCCCCA TATCCAACCA	60	(T)10	279
UN2676	TGCTGACAGACAC CAATGGG	GAAGAGGAGC TGGTAAGGCG	60	(CGG)6	166

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN2693	ACCTTCTGGTGGC TACCCTA	AACACACGACA ACACCACCT	60	(CCA)5	104
UN2725	TTTGAACGCCACA ACCAAGA	GGACATTCAAC TTGCTCGCC	59	(A)11	230
UN2741	TGGAACTCCTTGG GGTGTTG	ACTTAAAGTCA TGAAGCTTACA GGA	59	(T)10	268
UN2749	TCAGGGCTTGCAC TTGATGA	GGATGACCAGC GGGAATTACA	60	(A)22	260
UN2755	ACTTGGAGCGGAG GTGAATG	ACATCATTTTT GTCGAATGTGT GGA	60	(T)10	182
UN2756	TAGAGAGCACCTC GTCAGGC	AGTTTGGTGAA GGTCCAGGC	61	(T)10	259
UN2787	GCTACAAAAAGCG CGTTTGC	TCATAACACGT AGCGGCTCC	60	(CAC)7	209
UN2815	TGGCATTTAAGAT CAGGTCATCCT	TCTTGGTACAT ACTACATGTGT ACA	59	(A)19ctcg agactagttct c(T)17	260
UN2823	TCATGATACTGTG GGAAATGTGA	ACTGGAAAAAT AAATCATTGCT CAAGT	58	(A)27ctcg ag(T)18	209
UN2827	AGCAGAAAGCAC ATTGCACA	CAAAGGCTGGG AAGGCAAAG	59	(TAA)5	264
UN2882	TTTTCACTCTTTCA CTTCTCAACC	GCGGAGTCTGT TCGGAGTAG	59	(CT)6	155
UN2892	TGGCCAGTCTTTG TGCTAACA	GCAAATTCTGT AAAAGGCTACA CA	59	(T)10	259
UN2894	CCTCTACACGCTC TAGCTGC	TCTTTCAACAC ACACGCACG	60	(TG)6	279
UN2901	CGAATCGTTGCCC CGTAAAC	TTTGACTCGGG CAGCAGAAT	60	(T)12	271
UN2913	GGGGTTCTAGGTG GAGTTGC	CACATGCAAAT TTCACACGCA	59	(ATG)5	256
UN2957	TGGTTCCATTAGG GTACTGACC	AGAGCAACCTC AGCATCACA	59	(A)10	259
UN2994	ACTTGGGCTCCTA CGCAATC	TGGCTCGGGTT ATTTTTGGGT	60	(T)18	211
UN2997	ATCACGCACCGAA CCTAACA	CGCTAAACTAA ACGGTGCCG	60	(TC)6	272

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN3024	CACTTCATTCTTG GGCTAGGGA	TCCTCGTTCGA ATGATCCTGT	59	(GTT)6	181
UN3030	CAAAACCCAAACC CAACGCA	CGTTCCCAGCA TACCCTTGT	60	(CTT)5	245
UN3033	AAGCGCCGAAAG ATGAGACA	GGTTGCCTGGA ATTATCGGC	60	(A)27ctcg agactagt(T C)10	278
UN3044	ACAACACCATGAT GAGCCTTG	TGTGTCATCCA TGGACCACA	59	(GAA)10	256
UN3045	ACACAGAAGAAAT CAATGCATTGC	AGGCCAATCAG AGCTAGGGA	60	(TGA)7	149
UN3053	GAAAGAGAACTCG GGGTGGG	ACATCCCAGGG AAAAACAAACT G	60	(C)17	199
UN3074	GAAGACGGGGTTG CAAATGG	TGCAAAGACCA TTTAATCCGAC A	59	(A)10	275
UN3079	CCAAACTCTTCAC CGACACG	CGCCGAAAATC GCAGTGTAG	60	(TCTTC)5	155
UN3109	AACACCGGAAAA GAAAGCGC	GTACCGGAGAT CCAGCGATG	60	(AGA)5	141
UN3116	GCATTGATCTCTC CCGGGAG	CACCACGTTTT CCAGCACTG	60	(T)10	182
UN3119	CAGCCTCACCATC TCATCCA	TTGTGGTGTGG TTTCGTGGA	60	(A)10	207
UN3130	TGGGTTTCGTTTTG TATTTGTTTCT	TACATTCAGTG GGGCCCCTA	59	(A)18	227
UN3130	TAGGGGCCCCACT GAATGTA	GCAACAAAACC AAAAGAGACA GC	60	(AAC)5	112
UN3132	TCACTCCTCTCTCT TCTTCGC	TATCCGGTCTT CGTCCTGGT	59	(TCT)6	228
UN3149	ACTGTTCTTGCTTT TCCCACC	CAGCTTCTCTC CCAACCCTG	60	(T)11	156
UN3154	TGTGGCTCTATCTT CTGGGT	GGATGGACCAT GCAGCTTCT	59	(ATTC)5	242
UN3156	CCATACCAATGGG ACACCC	TCATGTCTAAG CCACTAAGGTG T	58	(A)12	167
UN3159	TGAACCAAAATGC ATGGGGC	CCTCCATCGTC ACCCTTAGC	60	(A)10	243

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN3169	CTCTCCCTTGTCGCACAAGT	CAAGAGGTTGCGCATTTGGG	60	(TCA)5	101
UN3176	TTTGCTTTTAGGCCGCCAAG	TCCCAGAATGAAGGGTTAACCA	59	(T)10	190
UN3198	CAAATGGCGGCATTATCGGG	TTCCTCGCTTCCTTTTGCCA	60	(TGG)5	277
UN3214	GAGGAAACGGGTAGGGCAAA	TCAATTGCGATCATGTTGCAGT	60	(AT)7	227
UN3216	TGTGAAGACGATGATGACATGGA	GAAGCACCAGAAAGCCTTGC	60	(A)18	246
UN3291	CAACCCATGGTGGTCTCCTC	CACGCGGAAAAGATTCAGCC	60	(CAAC)5	214
UN3299	GCCAATCAGTCCAGGACACA	CGCTCTGTAACCAAAGGAATGC	60	(A)21	275
UN3302	TGGCACCACCAAAGAGACTC	TGGGGTTCGAGATTGGGGTA	60	(CCT)5	246
UN3311	ACATGCCTGTGGTGGTTGAT	AGTGACACCATTTTCAGGGTCA	60	(GAT)6	272
UN3321	ACGACTCTGTTTCTTCCGCA	CCCTCCGGAAACTTCTTTGC	59	(CAC)5	253
UN3326	GGAGTTTCATGCGCCAAGTT	GGGCCCCGTCAAATGTAACA	60	(A)10	159
UN3328	TCTGAGTTGGGCGGAACTTC	ACATATCGGGCAACGCGTAA	60	(A)25	218
UN3346	AGCTTGGTATTAATTTGGACCGG	TGCCAACCCTACTTGGAACC	59	(T)14	100
UN3372	GCTCCCATCTCAGCAGTCAA	TGCATGTGTACGGTTAGTGCT	60	(A)18ctcgag(T)22	247
UN3375	AGCGCACATTTCATTTCCGT	GAAGCACCAGAAAGCCTTGC	60	(A)19	279
UN3409	ACTCTTTACATTGCTCTTCCACCT	TCGATCCTCGAACGCCATTT	60	(T)12	106
UN3414	CTCCTTCCATTTCTCTTTCTGCA	GACAAGGGTCAGCAAGGTGA	59	(TTC)6	200
UN3426	TCATTGCAGCTTCCAAACCC	TATACGTTGAGCGCGATCGG	60	(TC)6	129
UN3428	TGGACTGTACCAGGGTTGGA	CGTCTTTGGTACCAGCGTCT	60	(A)20	263
UN3431	ATGCACCTATCAGGGCGTTC	TGGGTTTGGAACATGATCATCA	59	(A)10	238

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN3433	GACGGATCTGAAGGCAGCAT	ACACTCAATCGCTTCCAGTT	59	(A)10	261
UN3444	TTGGACGGTTGGAATGGAGG	GACACACCCCTCTTCGAGTG	60	(ATG)5	158
UN3455	GCTTTGGCCTGAAAGAACCTG	GGGTTTCTTCACTCCTCCGG	60	(A)24ctcgagactagt(TC)6	230
UN3489	CAACATGCGATGAGGATTGTCA	GCTCATGACCACCTTTCCCT	59	(A)18	280
UN3497			0	(T)11	
UN3504	GCTCCATGAAGCAAATGGGTC	AGCTCCACCACAGCATGTAC	60	(GAT)5	145
UN3512	TGGATTGCTCGAAAGGACCC	TGAAGCATCTGGAACAACGGT	60	(A)12	275
UN3519	TCCCTTTTCTTCTTGACCGAGA	GTTCCGTTTACGCATGCGAA	59	(T)10ccgtattgtattttta catccaacttaattaaaaat cctaacaaac taaaaagata tttcaaaaat(A)10	267
UN3531	TCCATCTTGCCCTCAAAAGCT	AATGACCGCGGAGTGATTGT	60	(A)12	280
UN3548	GCGGTGGCAAACGTTAAGTA	AAGCAGAACCGAGCCAAGTT	60	(A)19	280
UN3573	AGGCGTCCTTTGTATGCACA	AACAGTCAACATAAACAACAGCGA	60	(GT)11	100
UN3579	CACCAGGGTCTCATGGACAC	GACCATACCCACCATGTGCA	60	(GCT)5	144
UN3611	CCCCAACCCCATTCTCTCAC	TTCTAAATCCGTACACTTTCCCT	59	(T)14	269
UN3641	ATATGCTTTGCTGGCGGGAT	GCACCAACAGCAGCATAAGG	60	(A)30	262
UN3652	CCGTTCAAGAAAGCCTGTGG	TCCAGATGATGCTGATGACCT	59	(AAC)5	214
UN3689	TAAGGAAGCTGGTGGTGCTG	CGACGGGAGAAATTTGACCG	60	(A)18	254

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN3701	TGGATCGATCAAG TTAGGGACG	ATGCTTCCACT ACCACTGCC	60	(GTA)6	240
UN3711	ACGGGTTGTTTTT GAAAATGGA	CATCTCCGCAA GATCCTCGT	59	(G)12	260
UN3718	TGGGTTCACTGTT CCAGAGC	AGCCATGAGAT TCTTCGAAGGT	60	(GTG)8	250
UN3720	CTCACTCACCCGA GAAACTCA	CTTCTGCGACG CAATGCTTT	60	(A)10	217
UN3728	ACTCGTCCACCAA AAATGAACG	GCACCACCAAA CTTAACTCCC	60	(CAA)5	235
UN3730	CCCCACCCTGTAG TTATGTCC	CCGAACGTTTT GGTCACGTG	60	(A)25	213
UN3749	CAGCAATATTCCG CGGGTTG	TCGCAGTGGAA TTAAACAAACA CA	60	(T)13	126
UN3767	GGGGTTTCTCGTG TGGTGAA	GCAAGCTCCTT CACTGGTCT	60	(A)18	259
UN3776	TCCAGGTAAACGA GAAGTTGAAGA	AGTGTGTGAAT TCGTGCCCA	60	(TATT)5	279
UN3814	TCGGTAGCTGCTA GTGTCAC	CTTCCACCACC ACCTTGACA	60	(A)11	248
UN3814	TTGTGCAGGGTCG ACCTTAC	GTCGATGTCCC AGATCAGCC	60	(T)13	211
UN3820	GCACCACTTCCAA ATCGCAT	ACGTTCCTCTG GTTCCAACA	59	(T)10	243
UN3849	GACGACTTCAGTT GAAACAGCT	TACCTGAAGGA GAGCGGTGA	60	(AG)7	205
UN3857	AGCCGCACAACAG TTTCAAC	CCCCTGATTGT TGTTGCTGC	60	(CAG)5	161
UN3863	GTTGCAGTTTTGA GGGCGAG	ATGTTGCTCAG CCCTTGAGG	60	(TTC)5	197
UN3884	GGAACGACAAGTA GTGCCGA	AGTGCATCGCT CATCGTCAA	60	(TA)8	274
UN3896	GGTGATTATGTAC ATGGGATGGGA	TGCTAGTACAC ACAGTGGAAG A	59	(TA)7	123
UN3966	CTTGTCCTCACCG GCATGAT	GTCCTACATTA TCCCATGTGAC CA	60	(T)11	193

Table A2. Details of 373 primers designed using Primer3 software based on 4513 lentil unigene sequences (continued).

Primer name	Forward primer	Reverse primer	T_m	SSR type	Amplicon size (bp)
UN3968	CACCCACCCACCAAAATTGC	CCGATCGACTGAAATCGCCA	60	(A)14ccatttctacataaagaatgatgaacaaaattg(A)12	265
UN3971	TGGGGGAAAACCAAACCACT	CCTTGCCAAGGGAAACATGC	60	(A)10	256
UN4009	AGTGCAAGAACATCGGTTGC	ACTAAGTCATTTCTCCCCTCGT	59	(A)10	275
UN4080	TCTGATACTTTCTTTGCCACTTCA	AATCCAGGTTCCCAGCACAG	59	(T)10	280
UN4086	CTTGTTGGCCGTTTTGGGAG	CTCCTCCAGTTGCAGCAGAA	60	(TGG)5	227
UN4086	GCTGCTGAAGCTAAGGAGGA	CGAATCGTGGATCAGGGACA	60	(A)13	270

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database.

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris46	AAAAATGGGCGAATACGAA	ATTTGAAGTCGGTGAGGTCG	59	(CCA)5	128
PUT187aLensculinaris99	GCGACCACTGTGTTGTTTGT	AGTCAATCCATTGTCTCCGC	60	(AG)10	191
PUT187aLensculinaris112	AATCATGAAGATCGATCCCG	TTCTCCCTCTGCAGCATTTT	60	(AAT)5	264
PUT187aLensculinaris153	GCATCACTGAAGTCAATGGC	AGTTGGGTCGTTGAGATTGG	60	(CCA)5	162
PUT187aLensculinaris195	AACGGTGTTCTTTCATTCGAG	CTGATAAAACGACCCGGAAA	60	(A)11	101
PUT187aLensculinaris214	AAAGCAAGAGGAAATCAAACTCA	CGTAGATTGCAGGTGAGCAA	60	(AGC)6	253
PUT187aLensculinaris238	GCTTCATCGTCGTTAATCGG	ATCGCGTATAGGATGAACGG	60	(CAC)7	193
PUT187aLensculinaris240	CGCAACCTTCTTCTGCTTCT	TGGATATGGTGGTGCATTTG	60	(T)21	108
PUT187aLensculinaris271	ATTCTCAAGTACGCGGCAGT	ATGAAGGTGAACGAGTTCGG	60	(TCT)5	272
PUT187aLensculinaris286	TTGCTTCCTGATGCATTTGA	GAATGTCGAAACCTGGCCTA	60	(T)10	273
PUT187aLensculinaris319	CCCTGCTATGCAACAAGGAT	AGCCTGCTGATGAAGTTGCT	60	(CAG)5	163
PUT187aLensculinaris355	CGTTTGATCTTCTAAGCCCC	TCGGCACATTGTTGAAAAGA	60	(T)10	194
PUT187aLensculinaris405	GCATCCTGAAAAGCAAAAGG	CGACAGGAAAAGCGAGGTAG	60	(ATA)11	279

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris471	TTATGTTCCCAGGCAAAAGG	CCACTGCCAGAAGATGAACA	60	(AGC)5	165
PUT187aLensculinaris479	AACCTCAGAAAAGAAACCCCA	AGGACCACAGGAAGAGCAGA	60	(CT)7	155
PUT187aLensculinaris525	TCAAGTCGATGAGGCAATTTT	CGGTGGATACCAAGCATAGG	60	(A)10	275
PUT187aLensculinaris533	ACACGTTCGTTTTCGCTTCT	CGAAAAAGACGTAGAAAAATCCA	60	(TTC)5	188
PUT187aLensculinaris535	TGAGTTTTCAGCAATGGCAA	GGACATGCCCATGTTCTTCT	60	(CAA)5	129
PUT187aLensculinaris545	CGGGGGAAGAAAAGAAAGAAA	GGGCATTGGAGAAGAACAAG	60	(TTC)5	189
PUT187aLensculinaris567	CAACGAAAACAGGGAAAAGG	CCCGTATCCTTTACTTTCCCA	60	(AG)6	267
PUT187aLensculinaris622	AAGTCCAAAAAGGTTGCACG	CAACTGAGGGGAAATGGAGA	60	(AGA)7	138
PUT187aLensculinaris624	GGTGGCGGAGAAGATTATGA	TTCTTCAATTTCCATTGGGC	60	(GTG)6	145
PUT187aLensculinaris647	TGCACCATCTTTTGCCTACA	CATAAAAATGATGAGCTACCTTCAA	60	(A)19ctcgag(T)22	143
PUT187aLensculinaris666	GCATCTGTTAGAAACACCAACAA	AGCAAAAGCAAAGGCAAGAA	60	(A)13	102
PUT187aLensculinaris666	TGGTGGTGTTTTGATGGCTA	AGCAATCACCTCACTGTGACT	59	(TTA)7	159

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris668	TTTTGCAGAGACGAGAGAAA	TCAGGATCGCATTGGTTGTA	60	(AG)9	234
PUT187aLensculinaris682	TCTCGCGTATACCTGCTGTG	CGGAAATCGTAGTTTTGGGA	60	(CTT)5	272
PUT187aLensculinaris694	CGCTCTAGCTGCATCTCTCC	CTTTCAACACACACGCACG	60	(TG)6	270
PUT187aLensculinaris716	TATTAGTGGGCGTGTGGTCA	CCCAATCTCCACTCCTCTCA	60	(GTG)5	137
PUT187aLensculinaris719	TAACTTTCGGTCATGCGTTG	TGATCCACTGAACTTCACGC	60	(GT)7	183
PUT187aLensculinaris760	ATTGGTGAATTTGGGGATCA	AATTTTCCATCATCCCCTCC	60	(GTG)5	176
PUT187aLensculinaris794	GTTCGCCACCAAAGACATTT	CTTTACGTCGTACCCCTCCA	60	(T)18	155
PUT187aLensculinaris862	CCCCCTTTCCTTAGAACTCG	TCCATTGAAACTTTTTGCTGC	60	(ATG)5	261
PUT187aLensculinaris887	AGAAGGCAGTGGGTGAAGAA	TCTAATCGCATCGTTTTCCC	60	(A)10	139
PUT187aLensculinaris889	GCAGCCTCTGAAGAAAGAGC	CTGCTTACCCACCACAACCT	60	(TGA)5	236
PUT187aLensculinaris930	AATCCATCTTGCCCTCAAAA	CGCGGAGTGATTGTGTTAAA	60	(A)12	276
PUT187aLensculinaris931	TGGGGTGTTGGTTTGTTTCT	TCATGAAGCTTACAGGAAATTACAA	60	(T)10	250
PUT187aLensculinaris1019	TTCCACTTCTGTTTGCACCA	ACGAACGGCTTGCTTTATGT	60	(CA)7	182

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris1052	TGGGTTG AATCAAG TTTGGA	TCCAGAAG GGCAGCTA AAAA	59	(TG)6	268
PUT187aLensculinaris1056	GAGCGTG CAGCACA ATTAGA	ATGAGACC CTCAACAA TGCC	60	(A)13	270
PUT187aLensculinaris1066	TCAGCTG GCTGTAC AAAGGA	GGGCATTT CCCTTTCTT TTC	60	(T)10	226
PUT187aLensculinaris1105	AGGAGGA GGAGGAT GTTGCT	CGCACTTC CAGACAAG TTCA	60	(TTG)6	103
PUT187aLensculinaris1196	CCAACCA TTTCAAC GCTAGT	TGACGGTT GCTGTTTG TTGT	59	(CCA)6	186
PUT187aLensculinaris1200	TTGTCAC TGTTCCA GGCTCTT	TTTGGCTT AAGAGATT CATTACTC A	59	(TA)6	171
PUT187aLensculinaris1231	TGTGGTA CATGCAC ACCAAAT	GGTGGTAG CAGTGGTG GAGT	60	(ACC)5	164
PUT187aLensculinaris1232	GCAGGCG TAGGAGA ACTTTG	TGAGAATC ACTTAACC CAAATGAA	60	(A)11	110
PUT187aLensculinaris1259	TCCAACA ATTCAGG CACAAC	AGGCTCCA GCTCCTAT TGGT	60	(A)10	124
PUT187aLensculinaris1263	TCACTAC CGGGAGA AAGTGG	CTACCCAC CACCTCCT CAAA	60	(TGG)5	115
PUT187aLensculinaris1271	GGAGAGA AAGAGAC GACAGGA G	TCGTTTTCT CTTCTGCG GTT	60	(AG)6	128
PUT187aLensculinaris1380	CACAACA AACCCTA GACGCA	ATCACTAC GCGTGTTG ACGA	60	(TCC)5	267
PUT187aLensculinaris1387	CAGCCGC AATAGGA AGACTC	CACAGAGC AGATCGAA ATCAA	59	(TC)7	220

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris1406	ATGACTG CCTCTCC AGCACT	CAGCAAAA TGAGCAAG TGGA	60	(T)12	239
PUT187aLensculinaris1454	GGAGTCG ACGAGTC AGAACC	TAATCTCT CCGGTCAC CGAC	60	(GGT)6	160
PUT187aLensculinaris1486	AAATGAG CATTTTG TGGAGTC A	TTGTAATG CGGCTTAG GCTT	60	(A)10	222
PUT187aLensculinaris1493	AAGGCAT TTGGTGG AATTTG	TGAGACAA TACCTGTTT GAAGC	58	(TAG)5	203
PUT187aLensculinaris1559	GAACAGG GGCTTTG ATGTGT	CAAGCTTA TCCCTCTCC ACC	59	(AGA)10	213
PUT187aLensculinaris1608	ATCCAAT CCCAATC CAATCA	CTGCTGTT GTTGTGGC TGTT	60	(CAA)5tt tcttcaacat (CAA)5	228
PUT187aLensculinaris1710	TGTCTGC TTAGGTG AAGCCA	CTACCGAA CGTTTTGG TCAC	59	(A)25	100
PUT187aLensculinaris1721	AAAAATC GCCACAA TCGCT	CCAGATGC ATTTGCCA TTTA	60	(TTC)5	241
PUT187aLensculinaris1782	TCCTTTC CTATGAG CACAAGT T	AGGGCACA TCAGTTTT GGTC	59	(TC)6	172
PUT187aLensculinaris1792	GACGGTT TAGGTTC GGTTGA	TTTTTGCCA CGCTTCTTC TT	60	(TTC)5	256
PUT187aLensculinaris1800	TGCCTAT AGGACGG ACAAGG	AGAGGGAG AGGAAGAC GGAG	60	(TCG)5	167
PUT187aLensculinaris1801	CCATGTT GTAGGGC AATGTG	CAACCCTC ACTTCCTC CAAA	60	(CAT)5	277

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris1850	TCCAATTCCCAGAAAATTAAAA	AAAGCAGCCTTGTTTGGAGA	59	(TC)9	273
PUT187aLensculinaris1855	TCCTTCCCCCTTTCTCATCT	TGAGGATGGTTTGGAAGAGG	60	(CAA)7	122
PUT187aLensculinaris1863	TTTCCCCTTCTATAAAATCCCTG	GTCACGGATCCGCTTAAGAA	60	(TAT)7	226
PUT187aLensculinaris1864	TTCGCGAACTCACTGTTGTC	TCTGTCATGCAAGGTCGTGT	60	(GT)8	268
PUT187aLensculinaris1870	CCACGTCATCAGCAAGAAGA	ATGGAGTGAATTTGAACCGC	60	(CCA)5	139
PUT187aLensculinaris1871	CACATTCAAAATCCACGACG	ACCGAGAGAAGAGAGTTGCG	60	(ACA)5	252
PUT187aLensculinaris1921	CACCCTTTTTCTGCATTTCAA	CTTGGGAAAGTGCAAATGGT	60	(ATA)7tccctttacag(CAA)5	241
PUT187aLensculinaris1925	ATCATCCCATGGCTTCACAT	ACTCCTCCAGCTGCTGACAC	60	(CAT)7	195
PUT187aLensculinaris1935	TCACATAAACCACAACAAGCAA	TCTTGCCTATGGCCAACATT	60	(AAT)5	108
PUT187aLensculinaris1979	TGAATCAAATTGGCATGGAA	CCGGTTCGGATCTTCTTACA	60	(AAC)7	175
PUT187aLensculinaris1991	CCGCAACAACAACTACACCA	GAAGGTTGTCCTTATGGCGA	60	(ACC)6actaccagtactacct(CCA)5	166
PUT187aLensculinaris2021	ACTAGGAAAGGAAAACGGCG	GAGTGACACGTGAATGGTGG	60	(TC)26	141

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris2033	ACAATCA GGTTTCG GACCAG	GCATCATC GATTTTGT GGTG	60	(CCA)8	246
PUT187aLensculinaris2042	GGCAGGA CCCTCTA TGGATT	CCACAACT CCCAACCT AACC	60	(A)30	265
PUT187aLensculinaris2096	TTGCATG TATGAAA CCGCAT	ATGGAGAA GCTAAGGG GGAA	60	(ATC)5	254
PUT187aLensculinaris2098	ACACCGA CGAATCC AATAGC	TTTGATGTT GAGGTGGA GCA	60	(T)19	222
PUT187aLensculinaris2104	ATTGCAG CCAGAGT GGAATC	AGAACGGC GTAAGCAG AAAA	60	(AAC)5	180
PUT187aLensculinaris2112	CATGACA ACGCAAC AGAACC	TGAAGAAC ATCTCGTG CTGG	60	(CAA)5	270
PUT187aLensculinaris2134	GCATCAT TACAGTG GTCCCC	CCGGCACT TCCTAATT CAAA	60	(T)12	277
PUT187aLensculinaris2168	TTGATGC CTAATAA TAACATG GTG	TGAAGATT TCATGCTG GTTTTG	59	(A)10	132
PUT187aLensculinaris2198	TGACTTC TCTGGTG GTGGTG	CACTTTGC CATCTCAA GCAA	60	(GGT)5	115
PUT187aLensculinaris2213	CGACCTT CAGAAAG CTTGATT C	CAACGCAG ACAACAAC ACAG	60	(AAC)5	265
PUT187aLensculinaris2239	CGTAGCT GGACTCT GGTTGA	CCTCGGAT AAACAAAA AGACAAA	59	(A)10	261
PUT187aLensculinaris2240	GCAAACA GTCACAA TCACCG	CAATCCAC AAGAACAC CCCT	60	(AGA)5	224
PUT187aLensculinaris2320	TGTATCA GTCCATT CACCGAA	CCTACGTT TCCTCGAA CAGC	59	(ATC)8	279

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris2408	TGGTATATGCAAGTAATAATGAAGTTG	TTAAAACCTGTATAGCAACCACG	58	(AT)7	157
PUT187aLensculinaris2414	GCAACATGGATTCTGGTGTG	AGCGAAAGATCGAAGACGTG	60	(CCT)7	252
PUT187aLensculinaris2431	GATTGCGGTAACCGAGCTAA	AGCAGTTTGTGACGACGCTA	60	(TGG)5	240
PUT187aLensculinaris2434	TGGAGTTGAGGCTGAGGACT	AGTTGCAGCAGAAAGTGCAA	60	(TGG)5	151
PUT187aLensculinaris2434	GCTGCTGAAGCTAAGGAGGA	CGTGGATCAGGGACAAACTT	60	(A)13	265
PUT187aLensculinaris2437	GCTGGCAATGTAGAAACAAAAA	ACAAAGGTGGAGCAAAGCTG	60	(AT)7	128
PUT187aLensculinaris2456	CGGGTTCTGTGCCGTACTAT	GGTCTTCCTCGCTTCCTTTT	60	(TGG)5	265
PUT187aLensculinaris2473	AGGAGCTAGAAGAGGGGCAT	GAAGCACGAGTTTCCTTTCG	60	(AAC)8	208
PUT187aLensculinaris2518	CAACATGCGATGAGGATTGT	GCTCATGACCACCTTTCCCT	60	(A)18	280
PUT187aLensculinaris2559	GCTCTCCCTGTATCCACCAA	ATCCATGCGAAAATCCAGAG	60	(AGG)7a agcattcgcagacgtctatcaagttcctccctcaacaacatccct(GCA)5	224
PUT187aLensculinaris2567	ATGGGCCGTAAAAGTGGTTA	AGGAATGGAGGAACGGAGTT	60	(CAA)5	252

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris2570	GCATTTC CGATCCA GAGAAA	GCTTGAAC TCGTCGAC AACA	60	(AG)7	204
PUT187aLensculinaris2576	GTCATGG ATCAACC CGATTT	CCAAATCA TCCACATG GTCA	60	(ATG)5	169
PUT187aLensculinaris2592	CAACCAA CCAAAGG CTTCAT	CGGATTGT GAGTGGGA AGTT	60	(A)11gaa tcgc(T)11	179
PUT187aLensculinaris2639	GATCACG CACCGAA CCTAAC	GTCCAAAC CCGAATCT TCAA	60	(TC)6	181
PUT187aLensculinaris2659	TCACCCG AGAAACT CAAACC	ATGATCTT CTGCGACG CAAT	60	(A)10	217
PUT187aLensculinaris2681	CTGGAGC CATAGTC ACAGCA	TCTAGGGC CAGAGAGT TCCA	60	(GAT)5	172
PUT187aLensculinaris2742	TGTCTCT GTTTTTA CCGTCGC	CTGGTGTG AGAACGAG CTGA	60	(CT)12	119
PUT187aLensculinaris2762	GTTGCCC CGTAAAC AATCAT	AAGCATCA GGCATAGC GAAT	60	(T)12	232
PUT187aLensculinaris2791	GAACACA TGTGGCT CATTCG	CCCCTTAA GATAGCCA GCAA	60	(T)10	197
PUT187aLensculinaris2815	CTGGCGA AAAAGAG GACTTG	GGCAAATG ATCTTTAG AAAATAAA A	59	(T)10	214
PUT187aLensculinaris2823	TTCCGCT TTCAATT CCATTC	GGGTTTTG CGGTTTAA GTGA	60	(A)10	269
PUT187aLensculinaris2852	AAATCGA CTTCGAA AACCCA	TAGGATTT TGGTTTCG CCAC	60	(CAG)6	179
PUT187aLensculinaris2874	TGGTTTT GGTGGTT GTCGTA	TGAAGCAG GAGTATTT GGCA	60	(AT)7	131

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris2913	AGATGCC ATACCTG TGGAGC	TGTGGTGG TGATGTTT GCTT	60	(A)10	196
PUT187aLensculinaris2969	AGCAGAA AGCACAT TGCACA	GGAAGGCA AAGGTGAA AGAA	60	(TAA)6	258
PUT187aLensculinaris2996	CCCAAAG CTCTTCC TCCTCT	ATGATCAT TTGGCTTTT GGC	60	(TCA)7	162
PUT187aLensculinaris3005	TCTGCAA CTTCAAC CACTGC	CATGGGTC GGTAGGTA ATGG	60	(AAT)5	124
PUT187aLensculinaris3102	GATTGGC CAGTCTT TGTGCT	GCAAATTC TGTAAAAG GCTACACA	60	(T)10	262
PUT187aLensculinaris3141	CCAAAAA TTTCCGC TGGTG	ATCATGTA GCACCAAA GCCC	61	(TC)6	229
PUT187aLensculinaris3167	GGTTACC ACAATGG TGGAGG	TTACCGTT GTTGGAAG CACC	61	(TGG)5	176
PUT187aLensculinaris3173	GCCATAA CGTTACT CACCCAG	GAGAATTG CGACGGAG AAAG	60	(GTT)5	128
PUT187aLensculinaris3192	TGCATCA TGTTACC ACCACC	GCTCTCGT GGTTTTCT GGAG	60	(TTC)5	235
PUT187aLensculinaris3192	CTCCAGA AAACCAC GAGAGC	GTTGCTGT TGTTGCTG CTGT	60	(CAA)5	264
PUT187aLensculinaris3201	TCTCCTC CTCCTCA TCCAAA	TTCAAAAC CTGAGGAC CACC	60	(TCA)5	124
PUT187aLensculinaris3228	CTTCCAA ACTTCCC AAGCAA	GATAGCGA GCCAAATG GAAC	60	(TTC)8	114
PUT187aLensculinaris3251	CTAAGGG TGGCCTT TGCTTT	GTGATTCC GATGCGTT TTCT	60	(A)19	111

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris3276	CGGAGGAGCATTGAGGTTTA	AATTACGGCGTGGAAAGAGA	60	(AGA)5	142
PUT187aLensculinaris3286	CCTTTGCACCAGTCATTTTG	TTGGGATTCAGAGAAATGGC	60	(A)25	224
PUT187aLensculinaris3338	TGGGTTTATTCTATTGCGGC	CGATCTCACCGAAAAGGGTA	60	(A)10	271
PUT187aLensculinaris3408	CCTCCCCATGAAAAGAACAA	TAAACCGTTGGTTCCAGGAG	60	(CAA)7	178
PUT187aLensculinaris3420	CCTAACCTTCACCACAAACCTC	TTGGAGAATGTGATCCGTGA	60	(TTC)5	225
PUT187aLensculinaris3421	AACCCCCAAAACCCTAACAC	CCATCGATTTCCTCGTTGTT	60	(AAC)6	188
PUT187aLensculinaris3424	GCGTGGGAAAACAAAAAGAA	TGAAGATTTGGGGGTGAGAG	60	(T)10	262
PUT187aLensculinaris3482	CCTAACCTTCACCACAAACCTC	TCGCCGTAAGACTGTCACTG	60	(TTC)5	181
PUT187aLensculinaris3510	AACAGCCAAAAGCTCCTGC	CACCATTTTCGATCAACCCT	60	(A)30	255
PUT187aLensculinaris3527	ATCGGAGGACCCCTTTTATG	AGTCCAAGAATGATCGGTGG	60	(CAA)5	225
PUT187aLensculinaris3532	TGGGGTTGAGTTCTTCAAATG	CCACAAATGTCACCAACACA	59	(ACAAGC)6	265
PUT187aLensculinaris3549	CTCCGTGGAAATAGATCCCA	CGATACGATCAAATCCAGCA	60	(T)10	137
PUT187aLensculinaris3583	TGGTGTGTTGAAGAAGACGAA	CAGCAGCAACAGAACGGTTA	60	(CAA)5	178

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris3589	TGGGAAAATCGAAAGAAATGA	CCGCGCCATTTAATAAGGTA	60	(CAA)7gaaggttttactactgctgctgaagaagaaacaacaacaa(CTC)6	238
PUT187aLensculinaris3602	TCTTTATAGTAGCAGGGGCAGC	GGCCGCAAAAAGTCAAATAA	60	(TCA)5	166
PUT187aLensculinaris3607	CACCCTCCTTTCCCTTGAAT	TGCTCGATAACAAGCAAACG	60	(CTT)5	248
PUT187aLensculinaris3660	AAAGAAATCGCCACCACAAC	CTGATTTTGGGTTGGGAGAA	60	(ATT)6	121
PUT187aLensculinaris3671	GGAAAGAGGGTGCAGAAGTG	GCATCACCGTGTTTGGTAGA	60	(T)13	245
PUT187aLensculinaris3717	TGACTTCCACACCTTGCAGA	TGGTCAGTGTTGTTGGCTTC	60	(TCA)8	229
PUT187aLensculinaris3734	TTGATGGGTTCACTGTTCCA	TTGTCCTTCAACCCTTTTGG	60	(GTG)8	233
PUT187aLensculinaris3743	CGCGGATACTATCTAGCCCA	TCGCTACGATGTTCTCGATG	60	(ACC)6	171
PUT187aLensculinaris3753	GTTCCTTCCTTGCTGCACTC	TTGAAGCGAGAATCGAGGAT	60	(ACA)6	157
PUT187aLensculinaris3798	ACCCACACACAAGCACAGAC	TCGATACGACATCCTCGTTG	60	(TA)6	256
PUT187aLensculinaris3800	CTCTTGTGGCTGAAGAGGCT	CCATCCAACTGAACGGATCT	60	(GAT)5	271
PUT187aLensculinaris3824	GTGTGTTGCACGGGTTAAAG	ACTCCAGTAGGTGGGTGTTG	58	(A)11	100

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris3835	CGGTAAAATATCACATTCTCTCCA	GTGGCGTCGGTTATCAACTC	60	(AAT)5	247
PUT187aLensculinaris3852	TATCTTTGCCACTGCCTCCT	TCCCGTTCAATATCTCTGCC	60	(TTC)5	256
PUT187aLensculinaris3875	AAATGCATTGATCTCTCCCG	TGCTCATCAAACACCACGTT	60	(T)10	197
PUT187aLensculinaris3877	TCTCGGTAGCTGCTAGTGTCA	CTGCGTTCGATTTGTTCTCA	59	(A)11	216
PUT187aLensculinaris3877	GTGGTGGTGGAAGAAATGCT	GATGTCCCAGATCAGCCTGT	60	(T)13	268
PUT187aLensculinaris3882	GTGGGAGGGTGTAGACCAGA	TTTCTCTCCAAATCCATGCC	60	(GTT)7	184
PUT187aLensculinaris3892	TTCCAGTAAAAATTCAAATTGATGA	GTTTTGGTGGTGGAAGAGGA	59	(A)10	261
PUT187aLensculinaris3952	AAAATGGAGGATTCGCAGG	GCTGCTACGAACATCACGAA	60	(GCA)5	135
PUT187aLensculinaris3989	GGAAACCATGGATTTGTTGG	ACTTTGTACCCTCCGTCCCT	60	(GA)6	108
PUT187aLensculinaris4022	TGTTCGACTTGATCTTTGCG	CGTTTGGACGAATGTCTTCA	60	(TAG)5	186
PUT187aLensculinaris4024	CAATGAATGATGCAAACCCA	TGGCAATTGTTCATGGTAGC	60	(T)11	246
PUT187aLensculinaris4027	ATGGACCATGCAGCTTCTTC	TTTCCCTTTAGAAGACAAATCCC	60	(TGAA)5	170

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris4063	TCCCTTTTCTTCTTGACCGA	GGGTATAAACCCACAACCGA	60	(T)10ccgtattgtattttacatccaacttaattaaaaatcctaacaaactaaaaagatatttcaaaaat(A)10	222
PUT187aLensculinaris4081	CACCCTTCTTCCATTCTCATTC	GTGCCGGTGGACTTACAGTT	60	(TC)7	206
PUT187aLensculinaris4111	CAGGACAAGTGTTGGTGTGG	AAATCACTGCATCGCATTACA	60	(TAT)8	237
PUT187aLensculinaris4114	CTGCAACGTTGAGTTTTGGA	GGACTGCCATTTTTAGAGTTCA	59	(T)13	271
PUT187aLensculinaris4158	GCAGCAAGAATGAACTGATTT	GAAGCAAGGTTGGTGTTGGT	59	(A)13	185
PUT187aLensculinaris4171	GCTGAAGCAAAACCAAAAGC	TCCACGGACGCACATTATTA	60	(GAT)5	179
PUT187aLensculinaris4240	AACCGCGTCTGCTAAGGTAA	CAGGAACAAGCGGAAGAAAA	60	(CAA)5	167
PUT187aLensculinaris4249	CAAGGATCTCGACCCATTCT	GTCCTTGCCGGTTGCTATAA	60	(AATG)5	273
PUT187aLensculinaris4305	ACCACCCATTTTTCTCCTCC	AGATTGTAGGGGGATGAGGG	60	(CCA)5	199
PUT187aLensculinaris4321	TATAGCGCGTATCCCCTCAC	TGTTGATGTGGCCAATTCTG	60	(TCT)5	227
PUT187aLensculinaris4340	TGGAGTTGAGGCTGAGGACT	AGTTGCAGCAGAAAGTGCAA	60	(TGG)5	151

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris4344	ACCCTTTTTCTTGCAAGCCT	CCTGTGGATGCCACTAGGAC	60	(A)17	271
PUT187aLensculinaris4365	TCTTGCGATGGTGACTCTTG	CCTAGCTATGGGCGTTCTGA	60	(TCA)5	151
PUT187aLensculinaris4395	AATCAGGGGTTGCAGTTTTG	AATGAAATGTTGCTCAGCCC	60	(TTC)5	211
PUT187aLensculinaris4416	ACACAAAGCAAAGAGCCACG	ATTTCTGCCGTTGGATGAAG	61	(A)10	180
PUT187aLensculinaris4511	TGTTGAGAGGAAAAGGGACG	GAGCCTCGATACTCCACCAC	60	(CT)6	278
PUT187aLensculinaris4530	GGAAGTTGAAGCGACGGTTA	TCTTTCTTCAGGAGAACCCG	60	(A)10	234
PUT187aLensculinaris4540	CATCAGCAGATGAATTGTTCCT	GCAGGTTGTTGTGGGAAGTT	60	(TTG)9	269
PUT187aLensculinaris4633	CGCTACTTCAGCTGCTCCTT	AAGATCTTGCTCCTCCCCAT	60	(TC)13	103
PUT187aLensculinaris4634	GAGGATGATGCATCCGAAAT	CAAAAGCTCTTGGTGTGGTG	60	(ATC)5	203
PUT187aLensculinaris4639	GGGATGGATCCCAAGTTTTT	GCACATAACAAAATGCAACGA	60	(T)10	232
PUT187aLensculinaris4675	GACTTTGGCGATCGTTGAAT	GTTGCTCTCAAACCAGAGCC	60	(GGT)6	214
PUT187aLensculinaris4681	CCTCCGATCTCCTCTTCCTT	TCGAAACAACCGTAGGAACC	60	(T)10	265
PUT187aLensculinaris4682	AATGGTCAAAAACTGCCACC	ATGAGCTGGACGAAGACGTT	60	(AAC)6	218

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris4683	TGCACAC ACAATGT TGATGG	AAGCCACC TTCAAAGC TCAG	60	(TTC)6	229
PUT187aLensculinaris4699	CGTTACC CAGGAAG TTGCAT	CTCTCCCTC TCTCTCTTG TTGTG	60	(CT)6	152
PUT187aLensculinaris4701	GGATTGG GAATGAA GGGTTT	GGCTGAGG CAAGTGTC TCTC	60	(GAA)5	124
PUT187aLensculinaris4747	AATCGGT GGGGGAG AGTAGT	CCCAAATC CTTTCACC AATG	60	(GCG)5	144
PUT187aLensculinaris4762	GGTCGGA GTAGCTT TCGATG	CGGGTCAG GTTGTTGA AGTT	60	(CAA)5	186
PUT187aLensculinaris4772	CCGTAAC GCTTCCA CAATTT	AAGCTGAT AGGGTCGC AGAA	60	(CT)7	188
PUT187aLensculinaris4820	GTTTGTA GGCGGAG GAATGA	CCAACACT CATTCGCT GAAA	60	(CGA)5	264
PUT187aLensculinaris4826	TGGACCC TAACGAA GCTGTT	CTGAATTG GGTTGAAC TTGC	59	(GGT)7	232
PUT187aLensculinaris4832	GCGTGTG AGGGTGA AGTGTA	CCCGTTCG TGTTTGTTT TTC	60	(AG)6	280
PUT187aLensculinaris4850	ACATAAG GACGAAA ACCCCC	GGTGAGGA CAGGACAA GGAA	60	(TCA)5	112
PUT187aLensculinaris4927	CGTAAGG AAGCCGA TGAAAA	AACAACGG GTCTTGAA ATCG	60	(A)10	162
PUT187aLensculinaris4955	GCTACCA TAACAGA CAAAACC C	CATGGCAA CAAAACCA AGTG	59	(AC)7	184

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris4960	CCCTCCA TCCCAAA AAGAAT	CTGGACCG ATCGACTG AAAT	60	(A)14cca ttctacataa agaatgatg aacaaaatt g(A)12	236
PUT187aLensculinaris4979	ACCCTAA ATCAGCA ACACCG	GAAACAAA CACACATC AACCTCA	60	(GAGAG T)5	200
PUT187aLensculinaris4992	CCTCGAT AACTTTC AAAACCT TG	TCAAAGGA GAACCGGA TTTG	60	(TTC)5	259
PUT187aLensculinaris4999	GTCATTG CAGCTTC CAAACC	ACCCTAAA TCAGCAAC ACCG	60	(TC)6	178
PUT187aLensculinaris5000	AGGGCTT TGTTTTG GGTCTT	TGAAAGCT TATTGTGG AGCTGA	60	(TGA)5	109
PUT187aLensculinaris5012	ATTTTGA TCCCAGG GAGACC	CTTCTCTTG GGCTTTGT TGG	60	(A)19	254
PUT187aLensculinaris5017	GGAAAAT TGTAGCG CAAGGT	GCAAGCGC AGAAGAAG ATTT	59	(T)10	279
PUT187aLensculinaris5032	CCACCAC TGTAAAG TAGGGAC A	GAGATAAA CGCCTTCG TCCA	60	(AGT)6	123
PUT187aLensculinaris5053	GTCGCAA TTCGCCA GTTATT	AAGATCAT GAGAAGGT GCGG	60	(TAA)7	180
PUT187aLensculinaris5069	GTTCAAC TTCCACA GCACCA	CTTCCTTCC CAACCACT TCA	60	(GAT)5	231
PUT187aLensculinaris5123	TATTGGG AGCGAAT CTGACC	TCCATTAC AAGATACT CTCCATGC	60	(TTTTA) 6	137
PUT187aLensculinaris5143	TTTCAAT CCCCTGA CTACGC	GCTCCGGA TTTTATTTG GGT	60	(AATCA A)5	163

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris5219	TGTCTTC CCAACTT GTTCCC	GGGAACTT GTCGATGT GGTT	60	(TTC)6	280
PUT187aLensculinaris5231	ATTTTAC TCATCGC CGTTGG	TCCTTATCC TTAGTTTC AAAACAGC	60	(T)20	277
PUT187aLensculinaris5284	TTACTTC GCCTTCC CATGTC	TTTGATTTT GCCTTGTG TGG	60	(A)19	228
PUT187aLensculinaris5315	ATGACAG CACCAAC CAAACA	TCTCTTCTC CATTACAC ACTCACA	59	(GAA)5t gagaagag agtaagagt g(TAT)5	274
PUT187aLensculinaris5370	TTACTTC GCCTTCC CATGTC	TTGTTCGC ATTGTTAA ATTTCC	60	(A)18ctc gag(T)22	168
PUT187aLensculinaris5371	CATTTTT CGGACTT TCTCGG	GGCATTTT GCTTTTGA GGAG	60	(A)20	212
PUT187aLensculinaris5375	ATCGCGT ATAGGAT GAACGG	CAGGAGAG TGACGGGA AAAA	60	(GGT)7	243
PUT187aLensculinaris5424	TCAAAGG ACACCAT CTATGCC	CTCCCTAA TGATGGAG GCAA	60	(AAG)6	210
PUT187aLensculinaris5457	AGGAGAT GCACTGG ATGCTT	AACACAGC TTCGCATC ACAC	60	(T)11	266
PUT187aLensculinaris5470	CAAGTTC TATGAGT GTTGGTA ACTATG	AAACTGAA AAGGGACC ACGA	59	(GTT)5	107
PUT187aLensculinaris5506	TGTGCTT AACGCCT CATCAA	TCCAACAA CCTCCTCTT TGG	60	(A)19	209
PUT187aLensculinaris5564	AAGCGTT GCAAAAT CCAAGT	GACTGTGC GTCAAATC AGGA	60	(GAT)5	105

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris5627	TGATTTTATTGATTCATCAACTCCT	GAGAAGCTTGTTGGTTTGGC	59	(A)12	196
PUT187aLensculinaris5634	GAATTGGCGTTGTTCTTGGT	TGTGGTGCAGTGGAAAAATG	60	(TCTT)6	237
PUT187aLensculinaris5655	CGCGCTGAATTGTACAGACA	GTATCGGAGAAGAAGCAGCG	61	(CAC)6	265
PUT187aLensculinaris5683	CCTAAAATCGACCCAAACGA	GACTCGGTTGGCATAGTGGT	60	(CAC)7	110
PUT187aLensculinaris5695	AAGAAAAGCCACAGAAGGCA	CTCATCATCCAAGCAGGGTT	60	(CAC)7	237
PUT187aLensculinaris5700	CTTTCCTCCCCATTTCCTTC	TGATGCGTGTTTTTGGTGTT	60	(TCA)5	100
PUT187aLensculinaris5723	TCTCTCTCCCTGTCCTTCCA	GGTTTGCCAAGTGGGTTTTA	60	(AAC)7	175
PUT187aLensculinaris5748	GCCTCAATAACTTGCGCTTC	TTGTTTGAAGGATTGCCTCC	60	(TGG)7	251
PUT187aLensculinaris5816	CATGCCTGTGGTGGTTGATA	TGACACCATTTTCAGGGTCA	60	(GAT)6	269
PUT187aLensculinaris5857	CAGGAAATGCAAGCTCCTTC	TTTAGGGGTTTCTCGTGTGG	60	(T)18	271
PUT187aLensculinaris5860	TCAGCAGCGATGTAAAGTGG	GACCTTGACGGGTTGAAGAA	60	(GAT)5	154
PUT187aLensculinaris5867	AGCATTGGGAGTGGAATGAT	GAAGCATCTGGAACAACGGT	60	(A)12	122
PUT187aLensculinaris5906	CAACGGTCGCTCAGTTAGAA	AGAATCACTTGGCGTTGGAC	60	(AC)6	157

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris5928	ATTGGAGGCTGAGAAAAGCA	TTGTTGTTGGGAATTTGGTGA	59	(ATC)6	270
PUT187aLensculinaris5933	GAAACCGCTCCATGTTTGTT	GCAAAAACAATTTGAAGGGC	60	(GGC)5	231
PUT187aLensculinaris6039	AAACCACGCCATCTCAAAAC	CATCGAATCCAACCTCCATT	60	(TG)6	202
PUT187aLensculinaris6119	TTTCTCTCACCTTGCCGTCT	CTGGTGGAGGTGGAGAAGAG	60	(CAC)5	183
PUT187aLensculinaris6130	TTGGCGATGTTAGTGATGGA	GAGGCACCCACTTTTTACCA	60	(TGA)6	255
PUT187aLensculinaris6201	CAGCTGTAAGGCACTGTGTG	GGTAGTGCTGGTGCTTCTCC	59	(A)27	240
PUT187aLensculinaris6240	TATTAGCGTTTGCGTTGCTG	ACCGATATCGTCACCGTCTC	60	(TTG)6	178
PUT187aLensculinaris6254	TGCCAGAATACTAAAATCATCATCA	TTGCTGTGGGGTAAAGAAGG	60	(TATTC)7	223
PUT187aLensculinaris6376	GCTTCATTGATAGTACAACGCC	TTGTGGTCAATGGTGAATCC	59	(AC)6	180
PUT187aLensculinaris6395	TGTTCGTGTCTTTCATCCCA	GAACTCCAAAATCCATCCGA	60	(CTAT)5	278
PUT187aLensculinaris6403	CCGGATACTGACGAGGTGTT	GAAAACCCACCATGGGTACTA	59	(CCT)5	167
PUT187aLensculinaris6420	TGTGAATATGTCTCACCCCG	AATAGCTTGTTCCACCGCAG	60	(T)10	236
PUT187aLensculinaris6427	CCACAATGGGAAGGTGATTC	AAAAACCAGCTGCGAACAAG	60	(T)11	275

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris6431	TCGCTCT TCTATTC TATCCCG	GAGCATGA AGACGGAG GAAG	59	(CT)8	277
PUT187aLensculinaris6448	TTCACCT CATAGAC CACTCCA	ATGGTGCT AGCATCTT TGGG	59	(CAT)5	173
PUT187aLensculinaris6457	TTTTCTC GCCGGAT TCATAC	CGCGAGAA GAGGAATC AAAG	60	(TCC)5	126
PUT187aLensculinaris6495	GGATGGT GAAGAGG GAGACA	AGTCTGAG GCGGATCC TTTT	60	(GAA)8	270
PUT187aLensculinaris6504	GTCCCCT GATTGTT GTTGCT	GGGCAAGC TATACCAC CAAA	60	(TGC)5	273
PUT187aLensculinaris6504	GTCCCCT GATTGTT GTTGCT	GGGCAAGC TATACCAC CAAA	60	(ATT)5	273
PUT187aLensculinaris6505	AGAAGCA GCAGCAC CAATTT	GAAGCATT ATCTTTGG GGCA	60	(CAG)5	172
PUT187aLensculinaris6530	GGCACAC TAGGCCA TTGATT	GTCCACTC AACCCCAC AAGT	60	(A)13	209
PUT187aLensculinaris6531	GCAGAGA AAGAAAG AAAGAAA AGAGA	GCTTTTCA GCAACTTC AGCC	60	(TG)6	257
PUT187aLensculinaris6533	TGAAATG CATGAAA ACACAGA A	GGCGGAGG TAATCTTG CATA	60	(TGA)7	213
PUT187aLensculinaris6541	CGGAATC AGGAAGA AGAAGC	TGTCTTTG GCGACTCT GTTG	60	(ACA)6	230
PUT187aLensculinaris6624	AGTTTCA TGCGCCA AGTTCT	GGGCCCCG TCAAATGT AA	61	(A)10	157
PUT187aLensculinaris6636	TTCTTTG CATTTGC TTGCAT	AGCAAACA TTTTCACTG GGC	60	(TC)6	117

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris6654	TCTGGGT TTCCTCG TTTTCA	ATGGACCA TGCAGCTT CTTC	60	(ATTC)5	227
PUT187aLensculinaris6665	CACATGA AAAGGAG GTGAAGC	ATGTGGTT TGGCATTG ATGA	60	(T)12	232
PUT187aLensculinaris6689	ACCGCAA ATCATTG GAAGTC	TTGATTTTG CTAACCCC ACC	60	(CTT)5	222
PUT187aLensculinaris6706	ACCGTTC AAGAAAG CCTGTG	TGCAAATT GGAACCAT AGCA	60	(AAC)5	163
PUT187aLensculinaris6764	TTGGAAT GAAAGAC CCTTGAG	GATGCAGA TGCTACCG TTGA	59	(CT)6	224
PUT187aLensculinaris6778	TCTCTTCT TCTGGCC TTCTCC	GGCGAATG CTTCTCTG GTTA	60	(AAT)6	274
PUT187aLensculinaris6783	AGATGCC CCAGTTT CAGATG	TAATGGGT TTTGGGAT TGGA	60	(AAC)5	144
PUT187aLensculinaris6820	TCTGTGC ATGGCTT TCTTTG	ACCCCAGT CTATCACT CCCC	60	(TGG)5	272
PUT187aLensculinaris6840	GTTCAGG GTGCGAA ATCAAT	GAAGCATG TTTACGGT GGCT	60	(A)27	262
PUT187aLensculinaris6872	ATATGGG GGAAAAC CAAACC	AGTGACAA AGTTGGGG ATGG	60	(A)10	212
PUT187aLensculinaris6925	TGCAGCA TCTTCAA CACCTC	ATGTGGAG CAAAGTTT TCCG	60	(TCA)6	242
PUT187aLensculinaris6962	TCCGCCA TCGAAGT CTTACT	TGAAAAAG GGTCAGTG GAGG	60	(ACC)6	223
PUT187aLensculinaris6971	ATGCAAT GAATTGG CCGTAT	TGTGGATA GTGGCCAT GAGA	60	(GCT)5	201

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris7092	TATTGGC CAGTTTT TGGCAT	TGGAAAAA TAAATCAT TGCTCAAG	60	(A)27ctc gag(T)18	271
PUT187aLensculinaris7106	TGTCTGG CTTGAGC AGAAGA	GGAAGCAT GTAACCCT TCCA	60	(A)22	214
PUT187aLensculinaris7142	TAGCGAC GGTTTTT GCTCTT	GAGAAGAA AGCATTGC AGCC	60	(A)26	221
PUT187aLensculinaris7232	CAGATAT AGCAAAA TATTCCC TCCA	CAATCACC AGTGGCTT CTCA	60	(ATC)5	129
PUT187aLensculinaris7255	ATTTTGT GTGTGTG CTGGCT	CCTACGCA TTTATAGC AAAGGAA	59	(T)10	153
PUT187aLensculinaris7284	AATAGGG TCAGGTG GTGGTG	CCCGTATC CATGTTAC CCAC	60	(CAC)7	226
PUT187aLensculinaris7376	TGCCCTC GATTTCT ATGGAC	TTGACCTA TCCGATGA TGATG	59	(TCA)7	171
PUT187aLensculinaris7379	CTACAGC ACGTTTG CAAGGA	TGGTCCCA TCCATAGC ACTT	60	(A)11	280
PUT187aLensculinaris7389	CGCGAGA AGACAGA AAACAA	GGTCGGGT TGATAAGG GATT	60	(GAA)5(AAG)6aa aacaaggg ccttccgct ccctttcag cgaatgggt ggaagatg gataaaaac actctctccc ttttatataac a(ACC)5	272
PUT187aLensculinaris7407	TGGTATT AATTTGG ACCGGC	TCACTTCA TCATTGCC AACC	60	(T)14	108

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris7416	GACTGTAACTCCTTCGCCCA	GCTCGGGTTATTTTTGGGTT	60	(T)18	231
PUT187aLensculinaris7475	AATGGCTTCGCTGACACTTT	CTCTGAGCAGCAGTAGCAGC	60	(TTC)5	132
PUT187aLensculinaris7478	ACTGGCGGTACTCACCGTTA	CCCATTGCTTCCTCTTGTTT	60	(A)25	280
PUT187aLensculinaris7488	TGGGTTTCGTTTTGTATTTGTTT	TGGACTCCATCCATTCTCAA	59	(A)18	206
PUT187aLensculinaris7512	ACCGTACGGATCAAAATTCG	TGGTTCCAACCTTTTCGTTC	60	(AGA)10	223
PUT187aLensculinaris7540	TCTCCTTTACTCCACACACTTCA	GAGCACAGTTGTTCCAAGCA	59	(AGG)6	269
PUT187aLensculinaris7660	GACCGAGGAATACCAAAGCA	TTTCATGCACTTTTCCCAAA	60	(ATG)5	280
PUT187aLensculinaris7662	CCTCCAAACGCATCTCTCTC	CTGAGCTTCGTTCATGGTCA	60	(TC)7	108
PUT187aLensculinaris7671	GAGAGGCTCATCATCGTCGT	ATCTCGCTGCTCCACAATCT	60	(TTG)5	182
PUT187aLensculinaris7680	AGGATTGGGAGTGATAGCCA	CACCGTTCGTAGTGGAGTCA	60	(CA)6	217
PUT187aLensculinaris7732	CTAAGCCTTGTGTCCGGTTC	ACAAGGTTGAGACAGTGGGC	60	(TCA)6	214
PUT187aLensculinaris7747	CCCGAGTCCATTTCCTTTTA	GGAGGTGGATTGTGCATGTT	60	(A)19	160
PUT187aLensculinaris7750	ATCCAGCTGACTGAGCATTG	GGTGATGGAAAAGGAAGTGG	59	(A)21	189

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris7770	ATGGTGC GGTTTCA AGGTTA	ACGATCAA AAGAAAAC CCGC	61	(A)21	276
PUT187aLensculinaris7803	AGGTAGG GATTTGG GATTGG	CGCGGAAT GATAGAGG GTAA	60	(TCT)6	185
PUT187aLensculinaris7814	TCAAGGG CTAAGAG ATGGGA	GTTGTTGC TGCTGTCTT GGA	60	(CAA)5	179
PUT187aLensculinaris7814	ATGTTAT GTGTGGC TGGGGT	CAACCACC CATTTGAA AAAGT	59	(A)11	235
PUT187aLensculinaris7900	TGACCCT GAGGAAG AAATGG	ATTGTGCG GAGGAAGA GAAA	60	(GAG)5	244
PUT187aLensculinaris7919	CTGGATT GGCTCTG GTGTTT	CCATGTTG TTTGTTTGT CGC	60	(A)18	208
PUT187aLensculinaris7927	TGGGAGA TGTCTGT TGGTGT	TTCTGCAA AAGCTTCT GGGT	59	(A)23	269
PUT187aLensculinaris7960	TACCTTG CAAACTC CGCTTT	GGAACGAT CTCGCTGA AGAC	60	(CTC)5	204
PUT187aLensculinaris8037	GTTGCTG TAGTAGC CGCCTC	AGCAGAAG GAGAGGGA AAGG	60	(ACC)5	250
PUT187aLensculinaris8041	GGAATCA AACCACC TTTCCA	CATCATTG ACCCATCA TCCA	60	(A)10	239
PUT187aLensculinaris8063	TTTCATC GTTCCAC AACACAA	GTGCCTTA CGGTGTCG TTTT	60	(AAC)5	151
PUT187aLensculinaris8066	GTTGCCG GCATTAT CTTCAT	CAAAACCA AACCATTC ACCC	60	(GAA)5	275
PUT187aLensculinaris8142	CCCTTTG TTTGGTT CATCTTT	GAACCCAT TCGCCACT AAAA	59	(CAT)5	143

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris8185	CCGTTTC TTGCTCT CGTTTC	GCGAAATT CTCCTAAC AGCG	60	(GTT)5	198
PUT187aLensculinaris8211	AGGGCAG CCTTAAT CCAGTT	TGATTTAT GTTTCGGA ACAACG	60	(AAC)6	258
PUT187aLensculinaris8336	TGAAAAC ATCAACT GTACAAA AAGA	GGGAAGGA CATGAAAG CAGA	59	(AT)6	278
PUT187aLensculinaris8344	ACCGCCC CAAAATC TACTTC	TGCGGCTC TTCTTTTCA CTT	60	(GAT)6	241
PUT187aLensculinaris8355	TGATTAT GTACATG GGATGGG A	GCCACAAA ATGCTAGT ACACACA	60	(TA)7	130
PUT187aLensculinaris8369	AAATCGC ATCCTGC AAATTC	TGTTCTGA AGATCGGG AACC	60	(CACT)6	202
PUT187aLensculinaris8392	CCACCAC CAATACC AGTTCC	CGGTGGTT TTTATGGA TTGG	60	(CCA)7	192
PUT187aLensculinaris8405	TTTGGAT AGTGATC CAGCAAT	AATCGCGT GCTTTGTTT TCT	59	(AT)7	207
PUT187aLensculinaris8410	GCAAGTC CAAAGAG TAGGCG	ATGGTCTG TTCACCAC CAGC	61	(A)18	264
PUT187aLensculinaris8415	GTGAACC TGGTCAT TTTGCC	TGCATCCC TTAACCCA TTTC	60	(GTG)7	274
PUT187aLensculinaris8420	CTACTTC CGCACCC ACAGTT	AGGGAGAT GCTGACAG TGGT	60	(T)16	256
PUT187aLensculinaris8422	TCAATTT CATAACC ATTCAAG CA	CGCACTGG CTACAAAG ATGA	60	(AAC)6	261

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris8429	CAGAGGT GCGATCT TTGCTA	CCCACCCA TAAAGCTC TCAA	60	(T)12	270
PUT187aLensculinaris8434	AAGCTTC GAGTTGC AGGAAA	CAAAGGAA ATCCAAAG GGAA	60	(A)10	246
PUT187aLensculinaris8440	AAACAGT TTGGAGG GGGAAT	ATCCATCA AGTGAAGG TGGC	60	(TTA)6	257
PUT187aLensculinaris8517	TCTTCTCT TTCAATC TCACCCT C	CTTCACAA GAAGGAGA GCGG	60	(CAC)6	250
PUT187aLensculinaris8533	CAACTTG CTCGCAG AATCAG	TGCCACTG ATGTAAAA CCCA	60	(A)18	245
PUT187aLensculinaris8561	GACGACT TCAGTTG AAACAGC TT	CTACCTGA AGGAGAGC GGTG	60	(AG)7	206
PUT187aLensculinaris8574	GCTTTTC CCACCTC TTTTCC	GTTGTACA CCGAACGA AGCA	60	(T)11	274
PUT187aLensculinaris8578	TCCCTTTT CACGTCA TCTCC	GCCGTAAC CTACACCT CCAA	60	(CAC)7	150
PUT187aLensculinaris8644	CTTCAGG GCTTGCA CTTGAT	CGTCGTTTT CCCTATGC AGT	60	(A)25	212
PUT187aLensculinaris8649	ATAATGG GCAACAG GTCCAA	CTTTGTGG TTCCAAAA TGGG	60	(A)34	146
PUT187aLensculinaris8651	CCTTCGT CACTACC CTGCAT	TACTTTGC AAGCACAG AGGG	60	(A)18	243
PUT187aLensculinaris8668	GGAAAGG CGTGCTA GAGAAA	GGAGAACA AGCCCCAT TACA	60	(A)28	272
PUT187aLensculinaris8669	TGTGAGG AAGAAGA TGCTGC	ATTTTCCC AGGGTGAA GGTC	60	(A)18	224

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris8702	ATCATCA AAACCCA AACCCA	GTTCCAAC TGTTCCCA GCAT	60	(CTT)5	259
PUT187aLensculinaris8705	TGATCCT GAGAAGC GTGAGA	TGAGCACA AGACATTC CTCG	60	(TGG)6	259
PUT187aLensculinaris8708	AAAGGGA CAAGGAA AATGCC	AGCCCTGT ACATCACC CAAA	60	(A)16	263
PUT187aLensculinaris8752	GGTTATG GAGGCTA CGGTCA	TCAACAAC CTCATTGT CGGA	60	(TGG)5tt atggtca(T GG)5	277
PUT187aLensculinaris8765	TAGCCAC CACTGGT TCTGTC	CTTATGGC GGAAGAAA CTGG	59	(TC)6	195
PUT187aLensculinaris8781	TAACTGC CCAGCTT TCTGCT	TTCACCCA TCAAAGCT ACAAAA	60	(T)10	198
PUT187aLensculinaris8811	TCATGAC CAGTCCC TGATGA	AAAAACCA TTGGATCC ACCA	60	(TTG)7	238
PUT187aLensculinaris8822	TTTCCTCT TTCAAGG GATTCAA	GAGCAACC TCAGCATC ACAA	60	(A)13	257
PUT187aLensculinaris8834	CAGGTGC GTGATGA ATATCG	GTATGGCG GTCATCGT CTCT	60	(TGG)5	266
PUT187aLensculinaris8849	CCACATT CTTCACC CACCTT	CTACTCCA CAGAGAAG GCCC	59	(TTC)5	166
PUT187aLensculinaris8857	CATCTCA AACTCCC ACAGCA	CTTTCGCA CCAATCAA ACCT	60	(A)11	128
PUT187aLensculinaris8888	TGCTGCA ACACGAT GGTATC	CAACCCGA TTTCGAAA AGAA	60	(CAT)5	239
PUT187aLensculinaris8888	TTCTTTTC GAAATCG GGTTG	GCAATCCG CTGAAAAT CAAT	60	(GGT)5	195

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris8915	TGTGGTC ATGGTGG TTATCG	CTCTCTCTG AGTGTTTC TGTCTCTG	60	(AG)6tgtt (AG)6	263
PUT187aLensculinaris8971	GCTGTAA TCCTTTC CTCCCC	TGCCAAGG TTTGCCTA GAAG	60	(A)18	229
PUT187aLensculinaris9004	AGCAGAA AGCACAT TGCACA	GGAAGGCA AAGGTGAA AGAA	60	(TAA)5	255
PUT187aLensculinaris9011	GCTGGAC AATCAAT TTCCGT	TACGATCC CTCGGAGA GAAA	60	(T)10	221
PUT187aLensculinaris9026	AAATTCG AATGCTT TTGGGT	GGTCGGGT ATTAGGTC CGTT	59	(AAT)5	228
PUT187aLensculinaris9031	CTATCAA GGATTTG CCTCGC	AAACTCCC ATTGATCT CATCTCA	60	(CTT)6	229
PUT187aLensculinaris9043	TCATTTT CTCCCAC TCCCAC	CCTTTGAA GGAAATTC TCAAACA	60	(AAG)5	223
PUT187aLensculinaris9044	GCGGAAC AAGAAAA CGTGAT	CACAAGTG AATTCTTA TTGCGA	59	(AGA)5	201
PUT187aLensculinaris9081	ATGAAGA TGATTTG GACGCC	CCTTCTTCG TCAAACGC TTC	60	(CCA)5	117
PUT187aLensculinaris9103	CAGCGGT GGTTAAC GGTATT	AACAACCC AATTGTTA CCGC	60	(T)10	252
PUT187aLensculinaris9137	ACCCGGT ACCTAAG ACTTCC	CCCACACA CTCTCCCA ACTT	59	(TC)15	146
PUT187aLensculinaris9141	TGAAGGG TAAAGGT GGATCG	ATGCTTCC ACTACCAC TGCC	60	(GTA)6	253
PUT187aLensculinaris9171	TTGGAAT CCATTTC TCTGCC	CAGGATCA GTCAGCGG GTAT	60	(A)10	259

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris9199	GCAGCGTAGTAGTAGTGATGGC	GCTTTTTCCGGAACGTTTTT	60	(TCA)5ctatcgctatcttccatttcaattccatttttctcaaaaaactcaaactcgccgcaatttcccttgcgtt(GCG)5	274
PUT187aLensculinaris9222	TCTCACACCACCAAAACCAA	AGGAGGAAGAGGCCGTAGAG	60	(CAC)6	258
PUT187aLensculinaris9224	CTCTCCAGTGAAGTAGCGGG	CTGGACCGACTTCAGAGAGG	60	(AGA)9	232
PUT187aLensculinaris9281	AACCTTCTTGGCAGCAGAAA	AACCTCCATTTCATCCATCG	60	(AGT)5	166
PUT187aLensculinaris9322	TCCGTGTCTACCTCCAAACC	GAAAATATGAGTCTGGTCGTTATGG	60	(CCA)5	173
PUT187aLensculinaris9351	CGAGAATTCGAACCCTGGTA	GGCCGTGAATTGAAGATTGT	60	(TG)6	276
PUT187aLensculinaris9364	CGTGTTCCCTTTATGGTGCT	TATGGCAACTTTTGGTGCAA	60	(GTT)5	227
PUT187aLensculinaris9382	TTACAATAACGGTGGCGGTT	TGTTGTACACCCCCACATTG	60	(TGG)5ttaccataa(TGG)5	215
PUT187aLensculinaris9405	ATTGGTGCACTCACCTCCTC	CAGGTGTTGGGGGAGATATG	60	(CCT)5	263
PUT187aLensculinaris9420	CATTTCAACCCCTTGCTGTT	TCCGATATAGCTCCGGTGAC	60	(CGG)5	175
PUT187aLensculinaris9442	GATGACGACGGCTATGACCT	CAGCAGGAACAAAGTGCAAA	60	(GTT)9	228

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris9444	TGAAGGTACTTGTTGCTGCG	TGGAGTGTGCATGTTAGGGA	60	(CAA)8	250
PUT187aLensculinaris9497	GCGTCATAACAGAATTCGTCG	TCAAAAGGGTCGACATCAAA	60	(CAA)5cggcttaaggagaaggttcttttcaatactagttttcttccgcttgttcctaagctcggaggc(GAA)5cgggaccgagagctacttgttgatattaatgagattggg(GGA)5	216
PUT187aLensculinaris9562	TCCCACCAAAGAATGGCTAC	TAAATCCCACCACCATGCTT	60	(T)14	264
PUT187aLensculinaris9701	ATTGGGATGCTGCATTTCTT	AAATGGTGCATGTGTACGGT	59	(A)18ctcgag(T)22	208
PUT187aLensculinaris9705	CTGCTTCCGAAATCTCATCG	GCGCCTGATACTGCTGTTTT	61	(GCG)6	135
PUT187aLensculinaris9711	CAAGGCTCATCAGGATTGGT	CCTTAGGAGAAGGTGGGTCC	60	(CAA)5	190
PUT187aLensculinaris9800	TTCTTCTCAATTCAGATCAACTTAAC	GCAAAACAGCCAGAGGAGTC	59	(A)11cataatagcatctattaaaacatacatgatggacaagcaatttctcaac(A)12	233
PUT187aLensculinaris9801	TCAACTAAGAATCGACCAAGCA	GGTTGAGATTTCTCGGGGAT	60	(A)10	220

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris9838	TGTGGACTGAACTGGACTTGA	CCGTTCGTTTGTCTGGATCT	60	(TAT)7	165
PUT187aLensculinaris9870	ATGTCAGTTCCTCCCAGCAC	GTTCTGCTGCTGATGTTCCA	60	(CAA)7	177
PUT187aLensculinaris9964	GCTTGGCTCAGACTGCACTT	GTCTTTTCCCATCGTTCCAA	60	(A)29	144
PUT187aLensculinaris9998	TGTCGCTCATTCCTTGTCTG	ACACCATGCCGTAAATGACA	60	(TTG)5	231
PUT187aLensculinaris9999	TGACATGTACAGGTTCTCAATGC	TTCCTCCCTTGATGAGGATG	60	(A)10	215
PUT187aLensculinaris10007	TCATCGTCATCCAAAAAGGA	AGAAGGGGAAGATGGGAGAA	60	(CAC)5	199
PUT187aLensculinaris10012	AACCCAATTCATTAGGAACGG	GAGTGTTAAAAGTCCGGCGA	60	(AAT)6	270
PUT187aLensculinaris10017	CGATTCAATTTGGGGAAACA	CCACCGTTAATCCCAACATC	60	(CAA)6	153
PUT187aLensculinaris10025	TTCCCTTCCCAATTTCTCCT	TTATGGAAGTGCGTGGTGAA	60	(CTT)6	138
PUT187aLensculinaris10026	GGGAATGCTATGCGATGTTT	TCCCACACCATTCTCTCTCC	60	(GTT)10	241
PUT187aLensculinaris10033	GACCAGCACACACAACAACC	TAACAACGATTGGACCACGA	60	(TCTTC)5	275
PUT187aLensculinaris10048	CCTCAGAATCCCACCATCAA	AAGCAAAACCCTCAACCCTT	60	(CAT)6	220
PUT187aLensculinaris10065	GCACCAGCATCCCAATAGTT	TGCTTGGACCCTAAATTTGC	60	(AT)6	255

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris10101	GACGCACTGATCGTAGCAAA	TCCAAATTGCATAACAAAACTGA	60	(T)10	232
PUT187aLensculinaris10196	CACACAATATACCGCCCGAT	CTTTGGGGCCGTTGTAGTAA	60	(A)20	198
PUT187aLensculinaris10202	TTCCCAGGAGCTTTATTCCC	TGCCTTTTGCAGGTCTTCTT	60	(CAA)6	248
PUT187aLensculinaris10257	GGAGCCATCATTGAATTCGT	GCTTGTATGAACCGCTATTGG	60	(TA)7	262
PUT187aLensculinaris10269	ATTTTCGCGACCATCAAATC	TTGACTGCGGAGGAAAGAAT	60	(CTC)7	126
PUT187aLensculinaris10278	CCGGTGGAGTTTTCTGTTATG	CATTCCCAGAATCTCAATTCC	59	(T)11	280
PUT187aLensculinaris10297	AGCTGTTGGATTTTCATGGC	AAAACAGGTTCTTTCTCCCG	59	(T)10	144
PUT187aLensculinaris10315	GATATGCATTGCGAGGGTTT	TCATTTATCTCGCGCTGTTG	60	(CGG)6	177
PUT187aLensculinaris10326	TAGCTTTGCCACCACACTTG	GCATCGGTTCGATTCTCAGT	60	(GA)6	104
PUT187aLensculinaris10344	TCACAAAACCTCAACCACCA	CAAACTCTCCAGCGGCTTAC	60	(GGT)5(GGA)5	175
PUT187aLensculinaris10354	TCTCCAGCGTCCAACTTCTT	GCAAGGAAGGGTTTTTAGGG	60	(TTC)5	196
PUT187aLensculinaris10408	GGTGCGGTGTTGTTGTATTG	ATTTAGAGATGTGACCGGCG	60	(GCGT)5atgtattcgtataatcggtgac(AGA)9	208
PUT187aLensculinaris10412	CCACCGCTAGTACCAAATCC	GGAGAGAGGGGAGAGAGGTG	60	(CAC)5	174

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris10444	TGCTGTTCATATTCGCTTGC	CGGGATGAATGGTGGTTATT	60	(TGC)5	167
PUT187aLensculinaris10462	AACTCCATTCTCATCCAGCA	CACTTTTGCTCACCAACCAT	58	(GA)8	280
PUT187aLensculinaris10463	GGAAAGAACAGAGAGCGTGG	ACACCCGCTTTCGACAATAC	60	(TGT)6	279
PUT187aLensculinaris10486	TTTAAGCAGCACCAAAACCC	ACACAGCAACTGGATGATGA	59	(TCA)13	126
PUT187aLensculinaris10492	ATGCTTTCCCCCTTTGAACT	CACCTGCTGGTTATCCTGGT	60	(AGC)5	242
PUT187aLensculinaris10513	AGCAGTGGAAGTGGCAAGAT	TCCAAAGTCCAAGCAAATGA	60	(A)10	225
PUT187aLensculinaris10531	TTTGCATTCCCCTTGATCTC	CACAAAATGCCATGGAACAG	60	(GTG)5	263
PUT187aLensculinaris10532	GCCTTCTTGTTCCCTGTTTC	TTTCTGATGGCCAATAAGGC	59	(T)11	153
PUT187aLensculinaris10533	TCCTCCAGGTCCAAAAACAC	CTACTTTTGCAACCCGATCC	60	(TTG)5	152
PUT187aLensculinaris10536	GTTGTTGCTGTTGCGTGTGT	GGTTGAACGGAGTGGAGTGT	60	(CCA)6	115
PUT187aLensculinaris10703	GGCATTTAAGATCAGGTCATCC	TGTGTACAATTGAAGTTATCATTTTG	59	(A)19ctcgagactagttctc(T)17	242

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris10708	GAAGCGCAAGTTTGGGATAA	CTCTCTTAATTATAAATTTTGGGATTG	59	(ATA)5aggttttgatttagcatgatgacgctaataatctatttgttctctttgagttttttgaatttttattttatatt(A)10	271
PUT187aLensculinaris10769	GCGGAGCAGGTAATTTTTCA	CGATTTGGGAAGAAGGATCA	60	(CAA)5	183
PUT187aLensculinaris10808	GACAATCCCTTTCACTCCCA	TCAACACTGTGAGCTTTCCG	60	(TC)6	142
PUT187aLensculinaris10834	AGATGATCGTCGGAAGATGG	TGAAGGGATGGTGTTGTTCA	60	(GTT)7	236
PUT187aLensculinaris10862	GGCTCTTATTTGACTTCAAAATTTCTT	TCTAACCAAAGCCCTCCTCA	60	(ATG)5	239
PUT187aLensculinaris10871	TGATCAGTGGTGGTGCTGAT	CGAGTCCAGTTCCGGAGATA	60	(CCA)5	125
PUT187aLensculinaris10881	CTTGCACTGCTTCAACCAAA	AGGCTACCAGATTTTGCAGG	60	(ATC)8	273
PUT187aLensculinaris10889	ACGACGAAGACGTTGATCCT	GCATTTCTTTGATTGCGATAAC	59	(TG)6	145
PUT187aLensculinaris10891	AACACATTTACAAAGACTCCAAAA	TCCACAAGGAGCACTAACCC	59	(A)10	239
PUT187aLensculinaris10892	CCTCTCAATCTTTCCGTTGC	CACCGTTAGGTCGAACGTCT	60	(TAG)5	267
PUT187aLensculinaris10897	TGAAGAGAAAACGGAGGTCG	GTGCGAAACTATGTCAGCGA	60	(AAG)7	224

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris10900	CTAAGCTGGAGGAGTGGCTG	GGAATGCTGGATCCTGTAGC	60	(A)13	201
PUT187aLensculinaris10947	CGAAGCACTTCAACCTCAAA	TCTGGTAACGGGATTTCTGG	59	(ACC)6	117
PUT187aLensculinaris10954	CCTCTCAATCTTTCCGTTGC	CACCGTTAGGTCGAACGTCT	60	(TAG)5	238
PUT187aLensculinaris10965	TGGTACAATTTCCTCTCCCC	TGGTCAAAGCTCAATTCCAG	59	(AGA)5	276
PUT187aLensculinaris10980	GCTTTTCCGAACTTCCCTTC	GAAATTCAAACCCCTCGTCA	60	(A)10	213
PUT187aLensculinaris10992	CCCCCTTTAGTGCAGTTTTG	CAATGGGAGAAGGCACATTT	60	(A)18	202
PUT187aLensculinaris10993	TGACCCAGAAAAGAAGGATCA	GAGCAACCTCAGCATCACAA	60	(A)10	241
PUT187aLensculinaris11000	AGTCTGAAGGTGGCGAGAAG	GCGGGTGCAGTTTGAGTAAT	60	(TAG)6	146
PUT187aLensculinaris11033	CCGTAACGGAAGTTGAAGGA	GCATCCAATACGACATGATGA	60	(GTT)5	143
PUT187aLensculinaris11107	GGATGGTGATTTTGGTTTGG	TGCAAAATCTGCCAATTCAG	60	(ACC)5	270
PUT187aLensculinaris11119	TTACAACCAAAATCGGAGGC	GTCGAAGAACTCGCCAAGAG	60	(TCA)5	207
PUT187aLensculinaris11139	TTTATATAGACACACACCCAGC	TCATCATGGAGATCAGCAGC	59	(AT)6	227
PUT187aLensculinaris11149	GCATGTCTAAAACACAACCCAA	TAGGCGTCCTTTGTATGCAC	59	(AC)7	225

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris11150	TAGGCGTCCTTTGTATGCAC	ACAGTCAACATAAACAACAGCGA	59	(GT)11	100
PUT187aLensculinaris11194	TTGCAATTTCAACTCACTCCC	AAATTCCTTGGTGCGATGAC	60	(TTC)7	138
PUT187aLensculinaris11209	GGATTGGAAGGAAATGCAGA	CCAATCCTCCTCCGTAACAC	60	(CTA)5	106
PUT187aLensculinaris11246	GCGATCCAGGTAAACGAGAA	GTGAATTCGTGCCCAAATAA	59	(TATT)5	278
PUT187aLensculinaris11247	CAATGGCTACGTGGATGATG	CCGCAATCCAAAACAACATA	60	(T)10	245
PUT187aLensculinaris11257	CACCATTACCGCATCCTCTC	AGCATGGTGATGAACGATGA	60	(TCT)6tccaaccgctctt(TTC)5	185
PUT187aLensculinaris11260	TCAGGTTTTGAAGGTGGAGG	TATCAGGCGAACCAAACTCC	60	(GTA)5	239
PUT187aLensculinaris11265	GAACCAGATTCTCATTCTCCTTTC	GCCATTAGAGATGCTGCTCC	60	(CT)9	151
PUT187aLensculinaris11267	ATCAGGTCTCGTTTGGGATG	GGACTTTGTTCGTTGACCGT	60	(ACT)5	277
PUT187aLensculinaris11279	TGATTTCACTCCATAAAGCTGG	GCGACAGACACAGCAAGAAA	60	(A)10	257
PUT187aLensculinaris11311	AAGATCGTTTGCTCATCTGGA	TGTGAGTTTTTCTTTGGGGTG	60	(A)18	269
PUT187aLensculinaris11313	GCCATCTTTCAGATTTTGGG	GATTGGCTGTGAAGCAATCA	60	(GAT)5	230

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris11425	ATTTTCC GATTTTC TTCCCG	CAATTTTCT GCATCATG AACCT	60	(TAT)5	274
PUT187aLensculinaris11432	GGGTGGT CAACAAC AGAACC	CAAACTGC GATGCTTG TCTC	60	(TGG)5	277
PUT187aLensculinaris11447	ATGTGTC ACAGTTG CTTCGC	GACTGCAA GGAGTAGT CGCC	60	(CT)19	242
PUT187aLensculinaris11460	TCTTTAC AATCTCA ACCTTCA CAGA	CATCACGT TGTTGTTTG ATTTG	59	(CAA)5a atgttgtctc tactggcctt cgtttatcatt cgatgatca acaacag(CAA)5ag attacagtta caacttcatc aatttcaatc t(CAA)1 1	274
PUT187aLensculinaris11470	CATTGCT CTTCCAC CTTTCTTT	ACAAGATT TTGCAAGG CCAG	60	(T)12	194
PUT187aLensculinaris11475	TTGGAAC GATCACG AAGATG	TCATGCCT TGAATTTG ATATGG	60	(CAA)6	221
PUT187aLensculinaris11545	TGGAAGA TGGGAAG ATGGAG	TGGCATCC AAAGCAAT TACA	60	(GAT)5	217
PUT187aLensculinaris11572	TGGATTT ACAAACA CGCAAAA	CAGCATGA CCCTGATG TGTC	60	(A)10	222
PUT187aLensculinaris11589	GCAAAAG TTCACGT GCTTCA	TTGTTGATT TAATGAAA GAGGAAAA A	60	(TTA)5	204
PUT187aLensculinaris11617	GATCCAC TTCATTC TTGGGC	CCTCGTTC GAATGATC CTGT	60	(GTT)6	184

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris11619	TGTTGAGGGATGTGAGTCCA	AACTTGGGTATGTTGGCTGC	60	(ATG)6	127
PUT187aLensculinaris11659	TCACTTCACCCTACTTCTTCTTCTC	AATCCAAATCAGCCATGGAA	60	(A)11	182
PUT187aLensculinaris11672	TTGTCGATATGAATTGCCGA	ACCACCTGCACCACCAGTAT	60	(GGT)7	130
PUT187aLensculinaris11686	TTGCAAACCTTCAACACCAA	TCCTCCTTCTCCTCTCTCCC	60	(GGA)8	260
PUT187aLensculinaris11688	CCCAAATTGTTAAGAAACCACC	TCGAGAACTGGGAGAGTCAAA	60	(A)15	279
PUT187aLensculinaris11700	CCAGAAATCAGATCTAGGGTTTTC	TCCCGAATCTACAATGGCTC	60	(AGA)5	277
PUT187aLensculinaris11712	CACTTTCACCATGGCTTCCT	GGGGGAAATGAGATGGTTTT	60	(TCT)5	168
PUT187aLensculinaris11740	CGAGGAATCTGAGTTTTAGAAGGA	TTGGAACGATCACGAAGATG	60	(TTG)5	226
PUT187aLensculinaris11750	TGAACACTTTCCCCTCACTCTT	TAGAGGACGCCAAGACAAGC	60	(TA)6	260
PUT187aLensculinaris11810	ATCACCGCTTCAAAAACCAG	GACGGTGATACCGAATGCTT	60	(ATC)6	148
PUT187aLensculinaris11823	CTGCTTTCGAATTGAGGTCAC	TGTGCCCCTACAAGTTCCTC	60	(ATG)6	143
PUT187aLensculinaris11825	TATTACTACAACGGGCCCCA	CAGATCCCATTTGCTGATGA	60	(TA)6	264

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris11830	CTTCTTCT ATACAGC GGCGG	AATTTGCG ATGATCGT AGGG	60	(AAC)6	165
PUT187aLensculinaris11904	GAGCTTT GTCTTGG TTTGCC	TTGTGCCA TCATGTTCT TCC	60	(GGC)5	252
PUT187aLensculinaris11920	GTTTTGG GAGGTGA TTTGGA	GCAAAGTC ACACTTGG AGCA	60	(TTG)6	225
PUT187aLensculinaris11946	TGGCAAC ACCAAAA AGATCA	CAGCCAAC CTCTTTGTT TCC	60	(CAA)5	243
PUT187aLensculinaris11966	AAAAACA TCCCCCA ACAGAT	TCCAGTTT GCAAAGGG AATAA	59	(A)18ctc gag(T)19	215
PUT187aLensculinaris11970	CCTCAGA TTGCACA AAAGCA	CTTCGTATT AATTCATC ATTACAAC AT	59	(TG)7	155
PUT187aLensculinaris12087	GTTTTGA GGGCGTG ATTCAT	AGCTCAAA TCGACCAC CATT	60	(ATA)5	221
PUT187aLensculinaris12098	CGGGGTC TAGCAGT CAACAT	CAATCCAA TCCATTTG GTCC	60	(TCA)5	262
PUT187aLensculinaris12122	CACACAC ACCCCTT CATCAC	TGTGCTGT GAATTTGG TGGT	60	(TTC)5	172
PUT187aLensculinaris12151	ATTCCAC GACATGG TCCAAA	CGGACTTT GGAATGGA AAGA	61	(GTT)6	189
PUT187aLensculinaris12167	CTCTCCT CCACCAC CTTCTG	ACACACGA CAACACCA CCTC	60	(CCA)5	115
PUT187aLensculinaris12213	AACCTTC CATGGCA CATGAT	TATTTAGT GGCGCCTA CGCT	60	(A)11	273
PUT187aLensculinaris12214	ACAACGT TAGGGTT CAAGCG	CCTCACTC ATACACTC TTTCTTTCA	60	(GA)6	119

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris12216	ATCCACTTGCATCCGAAAAC	TCTGGTGCAAGTCTGGTGAC	60	(ACA)9	174
PUT187aLensculinaris12222	TGATCGCTTTTGGGTTTTTC	AATTCTGATGCGGCGTTTAG	60	(A)18	249
PUT187aLensculinaris12223	AGCCGGAGAAAGTGAAGGTT	TTTGCAGTAGCTCTCATTCACC	60	(T)10	230
PUT187aLensculinaris12249	ACAGAAAAAGGAGGGGGAAA	TCCCTTCTTTACCAAACACCA	60	(A)11	183
PUT187aLensculinaris12252	GGAATTACAGAAAAACATGGTTGA	GAAATCTGGGAAGCAAGAACA	59	(T)11	280
PUT187aLensculinaris12267	ATTGACCAAGTCCAAATCCG	TCATTCCCAACATGAACCAA	60	(GTG)5	173
PUT187aLensculinaris12301	AATCAATCACCACATCTTAAAGAATA	CCAGTCCTGATCTGGGTCAT	59	(TTC)8ct(TTC)8	176
PUT187aLensculinaris12314	TGCCACTTCGATACTGGTGA	AGGATGCAAACCGCTGTATC	60	(CCA)5	250
PUT187aLensculinaris12328	TGCAATAACCATGTCGTCGT	TCTTAAGGCTCCTAACGCCA	60	(TCC)6	167
PUT187aLensculinaris12336	ACCTCTTTCCCTCGCATTCT	TTGCTGTTCCTTTTCGCTTT	60	(CTC)5	210
PUT187aLensculinaris12359	CGAGCCATGGATGAAGTTTT	GCGAAGGAGTCATTTGTTCC	60	(T)19catt aaaattcca aaatatattt cat(A)10	273
PUT187aLensculinaris12368	CACCACTGTTCCATACCCCT	TTGCTTCCCCCTAAAACCTT	60	(CTT)5	195

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris12425	AATGCAAATGGCACTTTTGTT	CTTCCTTTGCCTCCTCCTTT	60	(CTT)5	266
PUT187aLensculinaris12440	AAATCCAACCCTAACCCCAC	GAATTGAAGTTGGCGACCAT	60	(ACC)6	119
PUT187aLensculinaris12498	ATGGCATCAACGAAGGAAAC	CGGAGCAGAGACAAGAAACA	60	(T)12	154
PUT187aLensculinaris12560	TTTGAAAGGAGCAAAATGGA	TGCAAAATTGGGAACAAACA	59	(GAT)6	219
PUT187aLensculinaris12572	TCTCAAAAAGATCAAAGAAGGA	CCCAAAAGAGCAGTTCCAAA	59	(GAA)7	249
PUT187aLensculinaris12609	CTACCACCGGCCATAGTGTT	CACCTTCAAACACGTCCACA	60	(CT)7	101
PUT187aLensculinaris12629	TCACATAAACCACAACAAGCAA	AGAAGTGGCTGCTCTTCAGC	60	(AAT)6	254
PUT187aLensculinaris12639	CTAATATGCTTTGCTGGCGG	AACAACAGCAGCACCAACAG	60	(A)30	275
PUT187aLensculinaris12642	AGAGTTGAAGACGGTGCAAAA	GCTGTCACCGAGAATGATGA	60	(AG)8	136
PUT187aLensculinaris12670	TCATCAATTGGGCTGCAATA	CCTGGATAAACCGGTAGCAA	60	(TTA)5	235
PUT187aLensculinaris12691	TGAGACCCCCTAACTTTGGA	GTAGCCTCCTCCTCCTCGTT	60	(TTC)5	118
PUT187aLensculinaris12811	GTACCCCAACCCCATTCTCT	TTCTAAATCCGTACACTTTCCC	59	(T)14	272
PUT187aLensculinaris12813	CCGTTAGCTCTCTTCTCTCGG	TCCCTGTTTCGATATCAGCC	60	(CT)8	257

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris12882	CAACATTTGGGCTGGAAGAT	ACCCAAACCCACTTCAACAA	60	(TGC)5	153
PUT187aLensculinaris12903	GTCCGCCGTCAGTTAGAGAG	CCGCCTCTTCCTCATTATCA	60	(AAG)6	241
PUT187aLensculinaris12936	TCATTTCTGCTCCCTGCTTT	GGAGGAAGTGAGGTTGGTGA	60	(TC)6	159
PUT187aLensculinaris12960	CTATAACCGGCGAAAAAGCA	GATTCCGATCAGAGGAACCA	60	(GGC)5	102
PUT187aLensculinaris12979	AACCAGACCGTCACGTCTTT	GCCATTGAGGAGTTTGGTGT	60	(TA)6	198
PUT187aLensculinaris12993	CTTGAGGATCGGTGTTGGTT	CTCCGCCTCCTTTTCTCAAT	60	(GGT)7	171
PUT187aLensculinaris13013	CCAAATCAATCACATTTACATTTTG	AATTATCTGGAGGGGGATGC	60	(T)10	107
PUT187aLensculinaris13076	TTGATCGGTGATCAGATGGA	CCATAAGCATGAAAAACCGA	59	(T)10	238
PUT187aLensculinaris13101	TCCTAGATTTTCTCCCTCTCG	CCGAAAGACCAACTGTGGAT	59	(TC)7	199
PUT187aLensculinaris13175	ACATGGATGGACGGAACATT	AACCACCTCCACCACCATAA	60	(GTG)5	162
PUT187aLensculinaris13175	TTATGGTGGTGGAGGTGGTT	TGCACAACCAGATTCAGAGG	60	(GTG)5	259
PUT187aLensculinaris13197	CGTTTGAAAGAGACAACCTTTG	CATTCCCACCAAAGCAAGAT	59	(A)33	264
PUT187aLensculinaris13207	ATTTGGAGCAAAGATGCAGG	GGATCGACCTCCAATCAAGA	60	(A)10	198

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris13275	CAAGCTTGGATTCTGAGGTTTT	AATTGAAACAGAACGGGTGC	60	(A)19	271
PUT187aLensculinaris13299	TCAAGGCGGCTGTGTAATCT	CACCGTCAGTCGCACTAAGA	60	(AAT)7	226
PUT187aLensculinaris13302	AAGAGCTTTGTCAAACGGGA	CGAAATGATGCAATACGACG	60	(TTC)5	205
PUT187aLensculinaris13304	GCGAGTGCTGGTGTAGTGAA	ACCACCATCACACCATCTCC	60	(GAG)5	224
PUT187aLensculinaris13320	AAAAGCTGTTGATTTTGGCG	ACAGCCTGTTCCGAGAAAGA	60	(TC)10ta(TC)6	188
PUT187aLensculinaris13351	GGTTAAAAGGTGATTGTTTGCC	TGGGTAGGAACCAGCAAAAC	60	(T)10	204
PUT187aLensculinaris13363	GGACTAAAGTAACATGCATTCTGA	TTATTGGACACAGCGAGTGC	59	(ATT)6	237
PUT187aLensculinaris13383	TGTTCCGAATTGGATTGTGA	AAAGAAACGCGAAAACGAGA	60	(CAA)5	196
PUT187aLensculinaris13408	CATATCCACGATCCCTGCTT	CTATGGTGGTCGTCGTGAAG	60	(TCC)5	101
PUT187aLensculinaris13491	CCGTTGCAGCTTTAGCTTTT	TGTGGTCCATTAGGAGAGGC	60	(TCT)5	159
PUT187aLensculinaris13493	AGAGGCTCTTTTGCTTGTCA	AACCTTTGCTACCCTTGTCAAA	59	(A)10	153
PUT187aLensculinaris13527	TCTTCCTTGTCGTCACCTCC	ACGCGGGTGTCGTATCTAAC	60	(CT)11	273

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris13582	GATGAACCAAAATGCATGGG	GAAGGAGTTCGTCTTCGTCG	61	(A)10	184
PUT187aLensculinaris13614	GCAGGAGTTTAAGGTGCAGG	TTTACAATTTCATCATCATCAATATCA	59	(A)11	229
PUT187aLensculinaris13647	CAACAACGTCATCCTTCACG	AAACCCACCATTTTGACGAG	60	(CT)9	152
PUT187aLensculinaris13666	TAACAGATCGGTGCATAGCG	TCATTCCGATCGTCTTGTGA	60	(TC)8	151
PUT187aLensculinaris13718	AAAGACCAAGGCAACCACAC	ATACCTCACATTTCGCCGTC	60	(AG)6	268
PUT187aLensculinaris13724	CACTTGAGAGCTTTCTCCCG	GAATTTTCCGATTTTGCTGC	60	(TCG)5	193
PUT187aLensculinaris13758	CACTCATCCCTTGTTGCTCA	CATGAATCCGATCACCTTCC	60	(TTG)7	256
PUT187aLensculinaris13812	CGCTCTCGTAACCTCCACTC	TCTTCGGATATTTCATCGCC	60	(GAG)5	193
PUT187aLensculinaris13857	TATCGGCTGCTCCAACTCTT	GTGTCAACGAGGAAAACCGT	60	(CA)6	220
PUT187aLensculinaris13859	GTTGCGGATTGGTTGAAACT	GGATTACAATTACAAATTACAGACAGA	59	(GAGC)5gaggtagagagaaataaag(A)13	144
PUT187aLensculinaris13870	AGTCGTCAAAACCAGAACCG	AACGACGACACATCCTTTCC	60	(AGA)6	262
PUT187aLensculinaris13882	TGAGCTGTTTGGCAGAGAGA	CAAATGAAGCAAAACACGAAAA	60	(T)11	246
PUT187aLensculinaris13907	CCTCCTACAGAGAACAGGGC	CCAATGGATCGAAACCAAGT	59	(GTA)6	225

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris13957	CTCATAG GCCAAAA GGGTTG	GACAAAAC CCAGAAAA CCCA	60	(T)10	106
PUT187aLensculinaris13966	CGACATT GTTTTAT TCATTGC TC	GCGAGGAG GACAATGT GATT	59	(T)10	271
PUT187aLensculinaris13968	AGCCATG ACACAAC AAATCG	AGCCTTCT CCAGCAAA GACA	60	(A)19	279
PUT187aLensculinaris13976	AGGTTGG ACGATGA ATTTGG	CAGTCAGC AAGCAAAC CAAC	60	(T)14	163
PUT187aLensculinaris14052	CGTCTTG CACCTAC CCATTT	CCAAATGA AGGACCCG TT	59	(TA)7	138
PUT187aLensculinaris14074	AAGCAGA TCTCAAG GGTCCA	GTAAAGGG TGGAGGTG GGAT	60	(CAT)5	124
PUT187aLensculinaris14191	GTTATGG TGCGTCT CCCACT	TCAGGCAC ATGAAAAA TGACA	60	(T)10	265
PUT187aLensculinaris14213	CGTGCTT AATTTTA TAAATTC ATTTTG	GGAAGGTT GGGAAAGA AAGTG	59	(A)10	135
PUT187aLensculinaris14261	TGGATGC GAAGAGT GATGAG	AAACCACC ATGATGAA TCCG	60	(ACA)5	264
PUT187aLensculinaris14261	ATCGGAT TCATCAT GGTGGT	TGAATTGG ACTGAAGG GTCC	60	(TCA)6	263
PUT187aLensculinaris14273	CGGGAGT CTCTTCA ACTGTTT T	GAATTGTT TTGCAAAT CCGC	60	(TCT)7	123
PUT187aLensculinaris14278	CCCGAGA AGTGATT GTAGGC	CAGAGAAA TCCCCTGC TGAG	60	(CTT)5	273

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris14285	GGGTCGATGGTGAGACATTT	GGATAACGCAACTTCCCAAA	60	(GAT)5	201
PUT187aLensculinaris14288	GCGGTGGCAAACGTTAAGTA	GCGCTCCTTCACAAATTGA	60	(A)19	260
PUT187aLensculinaris14340	TGAAACTCAGGTTGTGGCTG	AGTTGGTAACCCTCGTGCC	60	(AAT)6	270
PUT187aLensculinaris14344	TGGTTTGCGTTTGAAGAAGA	GCATGAAAATTAGAAGCCTTGA	59	(GCT)6	141
PUT187aLensculinaris14386	CCAAAAGGGTACCATGCATTA	AACAATGAGAGGCCAGTGCT	60	(A)12	231
PUT187aLensculinaris14442	GATGCCAAATATTACGGTGGA	ATGGTTGTATCCTCCTCCCC	60	(GGT)5tacaatggtggaggaggaggctataaccatggtggtggaggaggatacaacca(TGG)5	192
PUT187aLensculinaris14471	AGGCACCCATGCATAAAGAC	CGAACGTGGTAACGTTTGTG	60	(ATC)5	232
PUT187aLensculinaris14475	TTTTACGTGAATGTGGCAGC	CAGGAGGAAGATGATGAGGC	60	(AAT)5	257
PUT187aLensculinaris14486	GAAGTTTTGTTCTTCTAATAGGGATGA	GGGATCCCGTGAATATTTTT	59	(A)10	276
PUT187aLensculinaris14486	CCAAATCATATTCGTTTGGC	TGATACCCTGCAAAGTGCAA	59	(A)10	274
PUT187aLensculinaris14499	AGGCTTCCAAGAAGGCTACC	TTCTCATCTCCTCCACCACC	60	(GGT)5	127

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris14527	AGGATGA AGAGGGT CCACCT	TACTCCCA AACCCTCC CTCT	60	(CTC)5	252
PUT187aLensculinaris14593	TGCCTTA ATTGTTC TTCCACA	TCCATTGA CCTTCACC ATGA	59	(CAAAA)6	200
PUT187aLensculinaris14596	CGTTGAA ACATTGG ATGTGC	AATGTGAT CAATGGTG GGGT	60	(AAC)5	261
PUT187aLensculinaris14623	AAGGATC AATGGTG AAGGAAA A	TCCATTTG AATGCGAT GATG	60	(CAT)5	158
PUT187aLensculinaris14636	ATCTTAT TCCTCCC GTGCCT	TCGAGAAA GGAGACCT GCAT	60	(CCG)5	119
PUT187aLensculinaris14651	AAGATGA GAAAACC CTTAAAT TTTG	CCAAAATA GTTCATTG AAAACGC	59	(T)11aaa attcaaaaa atgatgtga aataaacca (AT)8	173
PUT187aLensculinaris14651	GCGTTTT CAATGAA CTATTTT GG	TGGCGTTT TCAATGTT TGTG	60	(T)11	236
PUT187aLensculinaris14674	CCACGTC GATCTTC CTCTTC	GACCAAAA TCCTCAAC GGAA	60	(GCT)5	255
PUT187aLensculinaris14684	TCATTTC TCCACCG TTAGCC	CCATGCTG CTCCTGAT GATA	60	(CCT)5	101
PUT187aLensculinaris14711	CCATGAA TAAGGAG AACCGTG	CTGTAGGA AGACTTTG CCGC	60	(ATT)6	274
PUT187aLensculinaris14712	GATCATG TTCGGGG AAGAAA	CCAACACC ATCATCAA CCAC	60	(TGA)7	277
PUT187aLensculinaris14722	ACCCCTA AGGGTTC AAGTCG	TGGAAAGA AAAGCTGA AGGAA	60	(GAA)5	279

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris14731	TCATGCTAGAACAAACCCCC	CCTAGCTTTGAAGCTGGACC	60	(CTC)6	182
PUT187aLensculinaris14744	TTGGGTGATTTTGTTTGTGG	TGGCATTGCAAGATTCAGAG	60	(ATC)5gtatcgtccctaatta(TTC)6	254
PUT187aLensculinaris14792	AACATGTGCTTTCTTGCTTCA	TGAATTTGAGAAGTGCAGCG	59	(GGC)5ggagccttttctcctgctcctgctcctgctccttccccctgaaaataactg(TCC)5	269
PUT187aLensculinaris14800	GGTGAGGCATGTTGCCTATT	CCCCTTTTCAAATAACATTCTTG	59	(TGA)6	241
PUT187aLensculinaris14803	CAACACCTCACCACTCTCTCTCT	ACAGGCTGGCTCTCAACAGT	60	(CT)6	221
PUT187aLensculinaris14811	AAACACATAAGCCGGGACTG	AATTATGTTGGGCCATTGGA	60	(CCA)5	196
PUT187aLensculinaris14841	AAGCGAAATGGAATTTGACG	CAACCATCAACAGCATGACC	60	(GAA)5	246
PUT187aLensculinaris14872	TGGTGTGAGGATGATGTGCT	TTTTGATGTGTAATGGGTTTGG	60	(T)10	167
PUT187aLensculinaris14883	ACCACCCAAAACAAAACCAA	CCTTGGCAAAAACGACAGAT	60	(ACA)5	262
PUT187aLensculinaris14886	GTAGTGCCGAGAGAACGAGC	TCCCACATCATGTCAGGCTA	60	(TA)8	228
PUT187aLensculinaris14902	GCCACATACACACCTTGTGC	TGGTGCTATTCCCACTGTCA	60	(GTT)8	240

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris14938	TGCAATATCATCAGTTCACCAA	TGGAAATTATCTTCACCCCG	59	(A)21	279
PUT187aLensculinaris14996	CACCACTTCCAAATCGCATA	ACGTTCCTCTGGTTCCAACA	60	(T)10	242
PUT187aLensculinaris15004	CCATTCCCATTCCTTCCTTT	CCGTTGAAGTGGTCCTTTGT	60	(GGA)5	259
PUT187aLensculinaris15033	ATTCTGATGAACGGGGACAC	CTTCCTCCCTGTCATTTCCA	60	(TCT)5	118
PUT187aLensculinaris15073	CGGGAAAGGAATCAATCCA	AGATGGCGCAGAGACAACTT	60	(CAT)7	278
PUT187aLensculinaris15081	GGGGAGGCATTGGAAATTAT	GCCAATTTACATCCAATCCA	59	(AG)9	180
PUT187aLensculinaris15130	TGAAAACGAGCTGAAAAGGAA	TTTCACACCTGGAAACCCTC	60	(ATC)6	169
PUT187aLensculinaris15165	ATCGGAGAACTTGGACATGC	AGAATTGAAGCGCAAGGAAA	60	(GAA)5	170
PUT187aLensculinaris15207	TTGAGTTTGAGGGAATTGGC	CAGTCTCCTCCTTCGCTTTG	60	(AGA)6	112
PUT187aLensculinaris15303	AGGAGCTTTTTCTCTTGCGG	TCACATCTGAAAACATAAAAAGGG	60	(GAT)6	187
PUT187aLensculinaris15318	ATCATTGACGCCATTGCATA	TTCAAATGGGAAAACACCTTG	60	(T)10	275
PUT187aLensculinaris15360	TCAACCTTTCATCTCCGACC	GGGAAGAAGAAATGGGGTTC	60	(TCA)5	189
PUT187aLensculinaris15376	TGATGCTGCAAAGATGAACC	GCCACTTTGATGCTCCAATC	60	(TGT)5	257

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris15398	ATGGAAA TGGTGGT GTTGGT	CAACTACG TGTTCCGC GATA	60	(TGA)5	261
PUT187aLensculinaris15403	TCTGGAG ACCCCAT GTTACC	GCACAGGA TCACTCAG TTGC	60	(GT)6	257
PUT187aLensculinaris15446	ACTGCGA AGCCTCT CCACTA	TCAAGGTT CTCCCCAA ATGA	60	(A)10	240
PUT187aLensculinaris15518	TAGCTTC GCGGTAA AGGAAA	CACAGCAA AAACCAGC CTTT	60	(GAA)6	235
PUT187aLensculinaris15519	CCCATTG TTCAAGG AGGAAA	TCCATGAA AACGTTCG ATGA	60	(A)27	213
PUT187aLensculinaris15524	CAAACTA CCTCCAG CACATTC A	TTTAATAC TTTGTGGG GGCG	60	(AAT)6	125
PUT187aLensculinaris15540	AAAGACG GTGGCGT AAACAC	ACAGCCTG AAATGACC CAAG	60	(GGT)5	190
PUT187aLensculinaris15680	AGCGCTT GGTAAGA CGAAAA	TTGTCTCAT CATGCTCG CTC	60	(TGT)5	150
PUT187aLensculinaris15690	TTCATCT TCTGCCA AAACCC	GCCAGGAG TTGGAGAG TGAG	60	(CTT)5	137
PUT187aLensculinaris15690	TCTTTGG GTTTTGA CCAACC	TTCACGCA GAGTAAAA TCACG	59	(T)11	270
PUT187aLensculinaris15720	AGAAACC CGCAAGT AAAGCA	ACCTCAGA GCCGTTGT TTGT	60	(CTT)5	244
PUT187aLensculinaris15753	CCCGATT TGGAACC CTTATT	GGGACGAT GGTTCTTT GGTA	60	(TTC)5	112
PUT187aLensculinaris15768	TTTGCCC TAAGCCT CGTAGA	CTCATCTT GGACCACA GGGT	60	(AAC)7	278

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris15797	CAGGCTG ATTCCAT TGCTAA	AATCTCCA ACAAAACC GCAA	60	(T)11	238
PUT187aLensculinaris15832	ACATTGT ACTTGTT CGCGGC	CCTCAGGG GTTACAGG AACA	61	(TCAAC)5	157
PUT187aLensculinaris15847	CTGGTGA TTCTGGT CGGTTT	TATGTTGC GATTCGAC GATG	60	(TTC)5	154
PUT187aLensculinaris15848	CCATCAC AAACTTT TCGGGT	TGAACATA GATCCATC CACAACA	60	(TCT)5	167
PUT187aLensculinaris15856	CGTGAAG TGGTCCT GTTTTG	CACAAGTC ACCGATTG TTTCA	59	(CTT)5	134
PUT187aLensculinaris15875	ACAGCCT CACCATC TCATCC	GTTTGTGG TGTGGTTT CGTG	60	(A)10	210
PUT187aLensculinaris15876	GAACGCC ACAACCA AGATTT	AGTCACCA TTATGCCC AAGG	60	(A)11	245
PUT187aLensculinaris15887	CTCCCAC CAGTTGT TCCAGT	GTGCTGGT GGTGGATA AGGT	60	(CCA)5	190
PUT187aLensculinaris15915	CGGGAAA GAGTTAG AAGCCA	GATGCTGA TGCAATTG TTGG	60	(AGC)6	144
PUT187aLensculinaris15936	TCACAGC CATCACA GTCCAT	GGAGTTGG TGGAACTT TGGA	60	(CTT)5	125
PUT187aLensculinaris15943	ATGCTAA TGGTGTT GTGCCA	CCACCTTC GCTCATGC TACT	60	(TTG)5	201
PUT187aLensculinaris15954	CTTTGGC CTGAAAG AACCTG	GGTGGGGT TTCTTCACT CCT	60	(A)24ctc gagactagt (TC)6	233
PUT187aLensculinaris15976	TTCCTCT GATTGCC TTGCTT	TTGTTTGCT TCCATTCCT CC	60	(AGA)7	110

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris16055	TTCTTCCCAAAGTGGACCCT	TTCCTCCGGTGAGAATATCG	60	(CCA)5	196
PUT187aLensculinaris16072	CCTTTTCACTCTTTCACTTCTCAA	GCGGAGTCTGTTCGGAGTAG	60	(CT)6	157
PUT187aLensculinaris16105	TGGCCATAAGAGCTTCCACT	AAGCCAAAGCATTCTCAAACA	60	(AG)6	240
PUT187aLensculinaris16121	CAACTCGCATCCTCTTCACA	CAAAGGGGTTGGAGTCGTAA	60	(TTC)6	158
PUT187aLensculinaris16138	CTGGTGGTCTTTCACCCTGT	GGGAGAGGAGTTTGGAGACC	60	(TCA)5	202
PUT187aLensculinaris16203	CATTCGAATCATGTCTGGCA	TGCTGCTTATGGCAATTGAA	60	(TC)6	272
PUT187aLensculinaris16254	TCCCTAGGTGCATCCTCATC	CCTGATGGCTAAGGGTTTGA	60	(T)10	262
PUT187aLensculinaris16273	CAGAAACATTAGTTCCGTTTTGAA	TTGATGTGAAAGACATTTGTTCTG	59	(A)11	249
PUT187aLensculinaris16281	CACAAGTGAATTCTTATTGCGA	TGGAACAAGAAAATGTGATTACAGA	59	(CTT)5ctacttcagcttctgtaatcacgtt(TTC)5	239
PUT187aLensculinaris16321	ATTCCTGGATGGATGCTTTG	AACAGAGAAAAACACATGCAGC	60	(A)10	126
PUT187aLensculinaris16323	TTACCATACCAATGGGACACC	TGGAGACTCATGTCTAAGCCAC	59	(A)12	177
PUT187aLensculinaris16325	TCCATGTGCATCACCAGTTT	CAAACCCCATTTTTGAACAGA	60	(CTC)5	229

Table A3. Details of 658 primers designed using Primer3 software based on lentil unigene sequences in plantGDB database (continued).

Primer name/Unigene	Forward primer	Reverse primer	Tm	SSR type	Amplicon size (bp)
PUT187aLensculinaris16329	CCAAAGG CTTTTTC AGGTCA	CTCTTTTCC CCTGATCC ACA	60	(A)10	210
PUT187aLensculinaris16342	GTTTCTG GATCTTC CACCCA	CATGTTTC AATACAAC GCGG	60	(TGC)5	161
PUT187aLensculinaris16344	CCCATGA GCTGAGC TACCTT	TTGGTTTTG ATTCGGAG GAG	60	(GCA)5	277
PUT187aLensculinaris216350	CCCTACC TCTCGTT TTCCGT	CAGAAACC GAAGCTTC TTGG	60	(CA)6	241
PUT187aLensculinaris316350	TGCACAA CCAGATT CAGAGG	TTATGGTG GTGGAGGT GGTT	60	(CCA)5	258
PUT187aLensculinaris316356	TCTTCCT CCTTCCA ACGCTA	GGGAGAGA AAGAAAAG GGGA	60	(TTA)6	126
PUT187aLensculinaris316358	TTGTTCT GCTTCCT TGGCTT	TCAAACAA AGTCCCTT TGGC	60	(T)20	251
PUT187aLensculinaris416356	GGCTTCA TGAAAAT GAGCGT	GCCACTAG GCCAAGAA TGAG	60	(A)19	250
PUT187aLensculinaris816356	ACGGTGA GGTTGCT CGTTAT	AGCAGCCA CAAGCTCA AGAT	60	(A)11	275
PUT187aLensculinaris916358	TATCGGT TTGATGG GTGGTT	CAACACTG TTTTGTGG GTGG	60	(A)26	215

Table A4. Details of RNA quality data of the 36 samples of *Lens culinaris.*

Time course (h)	Treatment condition	Type of tissue	Replication	Absorbance at 260/280 nm	Absorbance at 260/230 nm
2	control	shoot	1	2.17	2.21
8	control	shoot	2	2.19	2.38
24	control	shoot	3	2.20	2.35
2	control	shoot	1	2.18	2.15
8	control	shoot	2	2.16	1.85
24	control	shoot	3	2.15	1.60
2	control	shoot	1	2.15	1.36
8	control	shoot	2	2.19	2.05
24	control	shoot	3	2.17	1.84
2	control	root	1	2.16	2.08
8	control	root	2	2.16	2.25
24	control	root	3	2.12	2.22
2	control	root	1	2.18	2.30
8	control	root	2	2.13	2.33
24	control	root	3	1.77	1.86
2	control	root	1	2.14	2.64
8	control	root	2	2.08	3.83
24	control	root	3	2.16	2.40
2	Excess iron	shoot	1	2.18	2.47
8	Excess iron	shoot	2	2.17	2.50
24	Excess iron	shoot	3	2.14	2.27
2	Excess iron	shoot	1	2.09	1.88
8	Excess iron	shoot	2	2.12	2.19
24	Excess iron	shoot	3	2.10	2.31
2	Excess iron	shoot	1	2.11	2.30
8	Excess iron	shoot	2	2.06	1.55
24	Excess iron	shoot	3	2.14	2.21
2	Excess iron	root	1	2.15	2.30
8	Excess iron	root	2	1.92	1.97
24	Excess iron	root	3	2.15	1.88
2	Excess iron	root	1	2.16	1.35
8	Excess iron	root	2	2.18	2.07
24	Excess iron	root	3	2.19	2.29
2	Excess iron	root	1	2.06	1.36
8	Excess iron	root	2	2.03	1.31
24	Excess iron	root	3	1.87	1.66

APPENDIX B. FIGURES

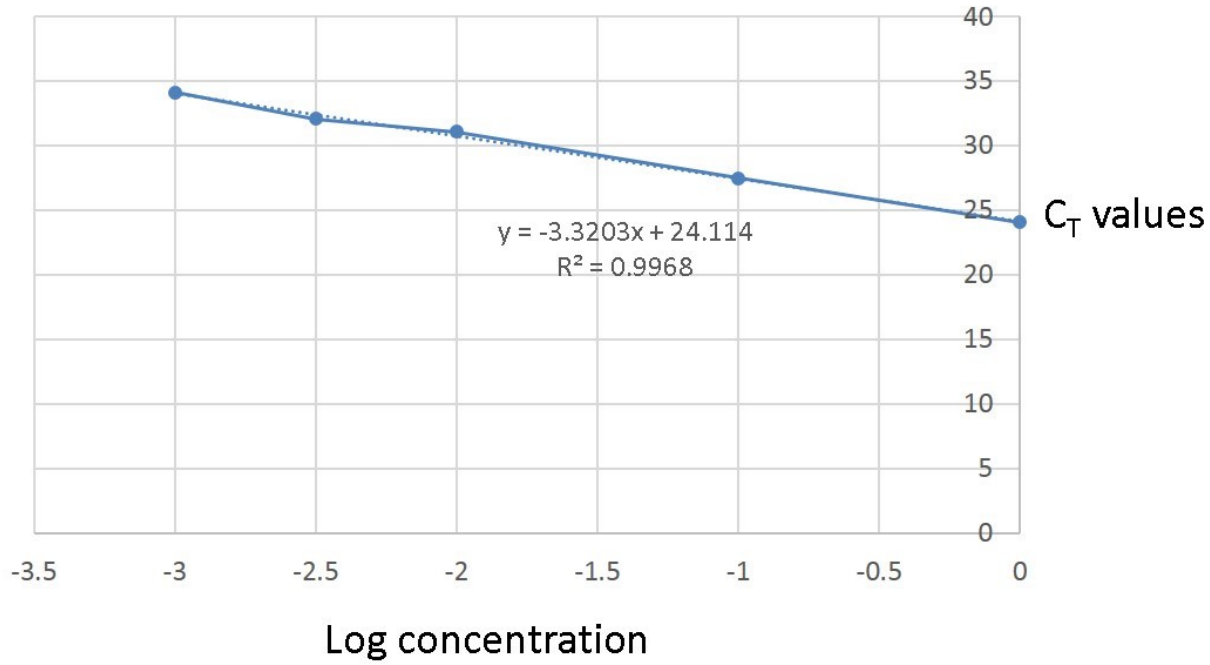

Fig. A1. Amplification efficiency of *Ferritin1* primer pairs

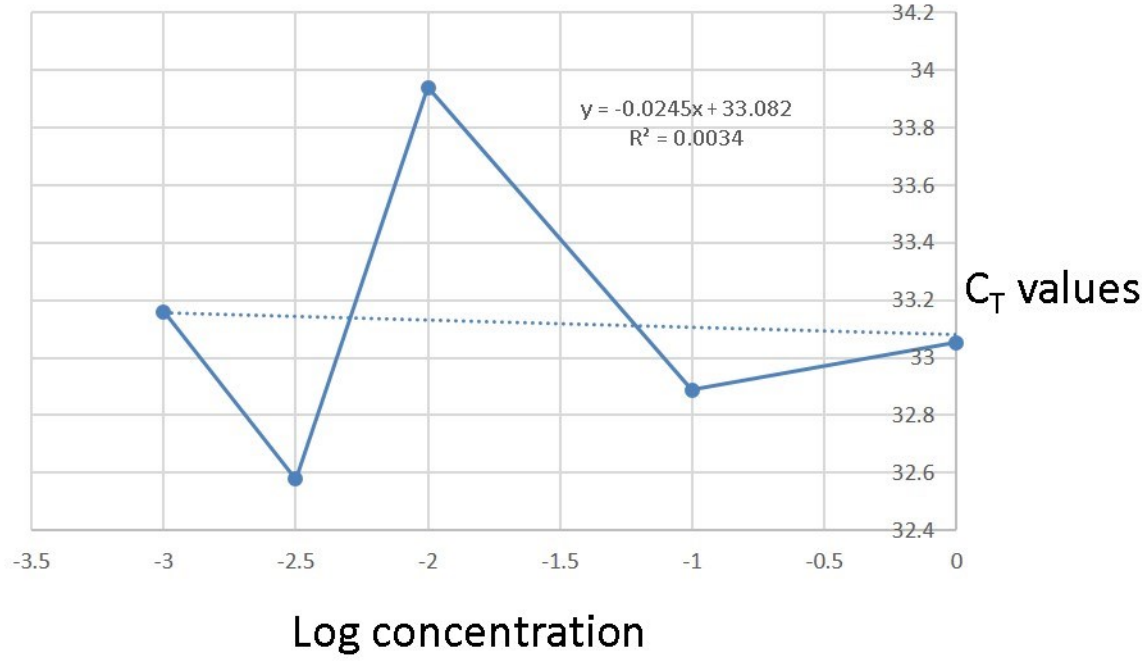

Fig. A2. Amplification efficiency of *BHLH1* primer pairs

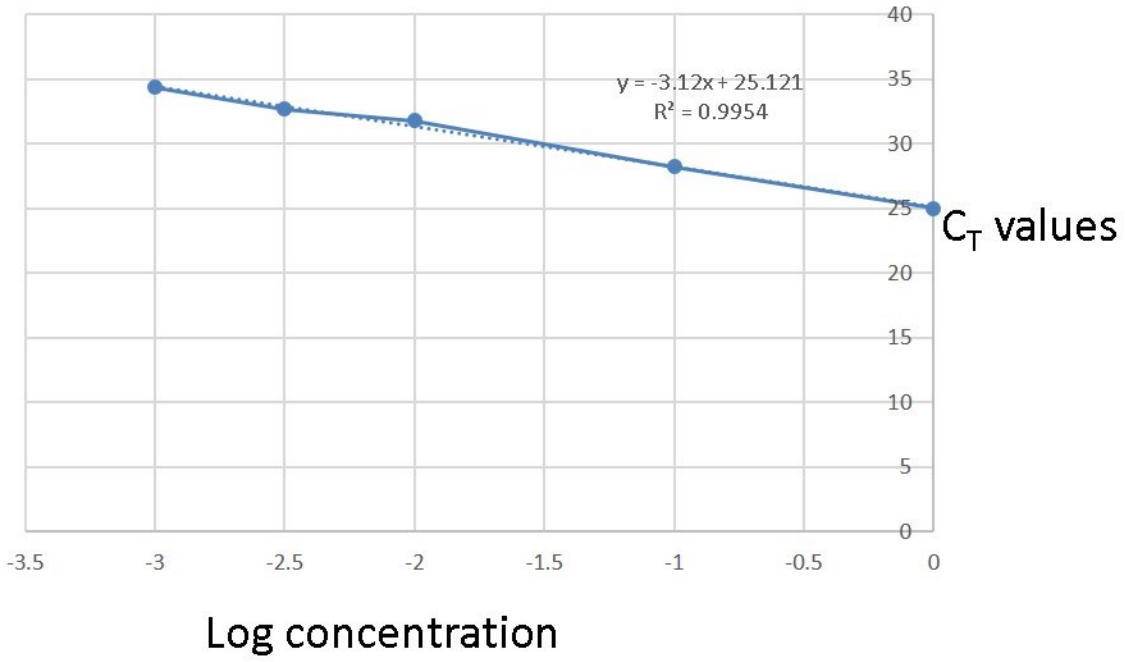

Fig. A3. Amplification efficiency of *IRT1* primer pairs

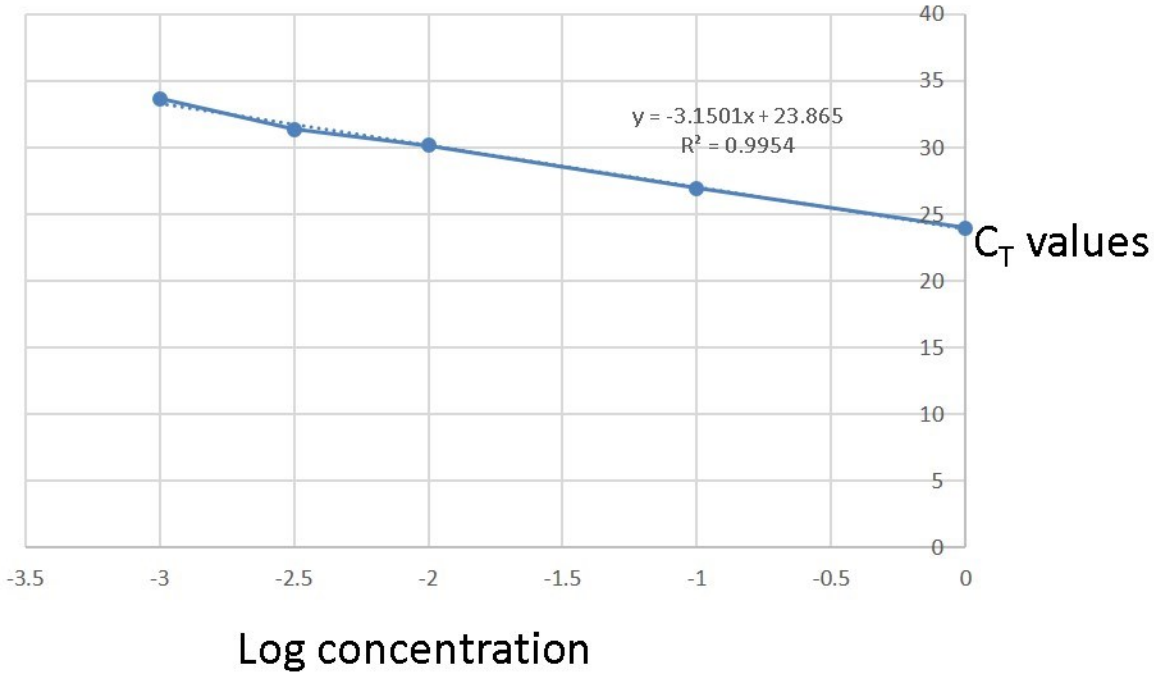

Fig. A4. Amplification efficiency of *GADPH* primer pairs

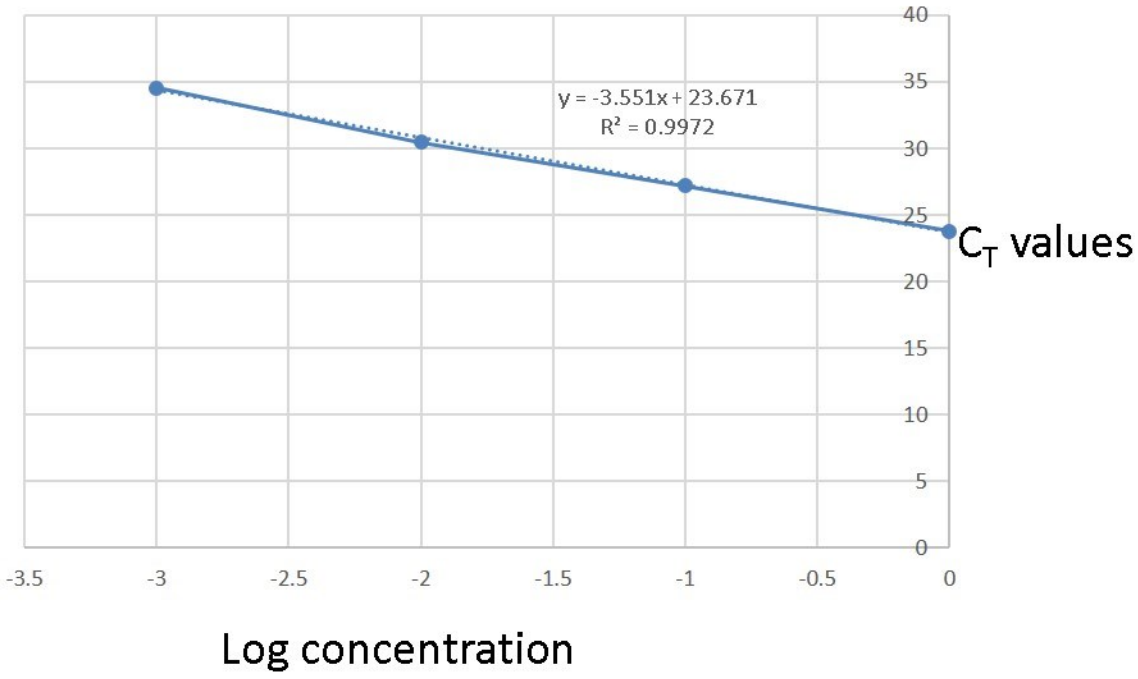

Fig. A5. Amplification efficiency of *Actin* primer pairs

APPENDIX C. LIST OF UNIGENE SEQUENCES

Transcript sequences for PUT series of markers were obtained from plantGDB database (http://www.plantgdb.org/download/download.php?dir=/Sequence/ESTcontig/Lens_culinaris/current_version)

>PUT-187a-Lens_culinaris-99 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

AAATACATTGTATTTCATTTCCCACGCGACCACTGTGTTGTTTGTTGTTGTGACCTGT
TTATACTTCATAACACAACTAATTCCTACTACAACTGATTCATACTATAAACAAAGA
TCACAACTTTCCAGAGAGAGAGAGAGAGAGAGCTTAGGTGGGTTCTTCTTCAATTTC
AAGAAACATGGATGTGGGTCAGATGCGGAGACAATGGATTGACTACATCAAACCCA
TGTTCACGGAGGGGTTCTTAGATGGTCAGTTTCTGCAACTTCAACAGCTACAAGATG
AGAATAACCCTGAATTTGTTTTTGAAGTTGTTTCTCTTTTCTTTGATGATTCTGAGAG
GATTCTCAAAGATCTGTCTTTTGCTCTGGAGCAGCAAAGTGTTGACTTCAAAAAAGT
TGATGCTCATGTGCACCAGTTTAAGGGTAGCAGTGCAAGCATAGGTGCAAAAGGGG
TGAAAAATGCTTGCATTGCTTTCCGCAACTTCTGTGAGGAACAAAACATAGATGCTT
GCCGCAGATGTTTACAGCAAGTGAAACAAGAGTACTTTGTTGTTAAGAATAAGCTTG
AAACATTGTTGAGGCTTGAGCAGCAGATAGTTGCAGCTGGCGGCTCGATCCCTATGA
TGGAACTGTGTTTTTAAAGTGAGTGATATGTAAAAATGCTTGTATTATGATCCTCTTG
TCTGTTGAGGATCATCTATTTCTCGTATGTTTCTTGTGAATGTATGAAATTTTATGTG
GATAGAAGTTTTCATCCAATGTACCTTGTTCTTCACAAGAAAACACTTTCATAATCTG
TAATATTTTTCATGCTTTAATGAAGGTTAATCGCACCAATATAAGATAGATTAGCTTT
C

>PUT-187a-Lens_culinaris-668 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).CACAACTTGCACTTCTTATACTTTTGCAGAGACGAGAGAGAAAGCAAGAGAGA
GAGAGAGAGAGAACCACCTCTCACTCCATCTTCACTCTCACTCTTCTACTGCCAGAC
CACCAGAACCTGCAATGGACTCGCGCACACAGTATAATCCTCGCACTGTTGAAGAA
GTTTTTAGGGATTTCAAGGGTCGTCGAGCTGGTCTCATCAAAGCTCTCACCACTGAT
GTTGAAGATTTCTACAACCAATGCGATCCTGATAAGGAGAATTTGTGCTTATACGGC
TTTCCTAGTGAGCAGTGGGAAGTTAATTTACCTGCTGAAGAAGTTCCACCGGAGCTT
CCCGAGCCTGTGCTCGGAATTAACTTTGCTAGAGATGGCATGCAAGAAAAAGATTG
GCTGTCTTTAGTTGCTGTTCATAGTGATTCTTGGTTGCTTGCTATTGCCTACTATTTCG
GAGCCAGATTCGGGTTTGACAAATCTGACAGGAAACGCCTTTTCAATATGATCAATG
AACTGCCATCGATATTTGAAGTTGTTACTGGTACAGCAAAGAAACAAGTTAAGGAA
AAGTCTTCAGTTTCAAACCACAGTGGCAGCAAATCCAAGTCTAGCTCAAAAGTGCG
AGCTCCAGAACCTCAGGTTAAGCAGACAAAGGCATTAGAACCTAAAGACGAGGAAG
AAGAACTGGATGAGGAAGATGAAGATGAACATGGAGAGACCTTATGTGGTGCATGC
GGTGAGCATTATGGAACAGATGAATTCTGGATTTGCTGCGACATCTGTGAGAAGTGG
TTCCATGGGAAATGTGTGAAGATTACACCTGCTAGGGCTGAGCATATCAAGCAATAC
AAGTGTCCATCATGCAGTAACAACAAGAGAGCTCGCCCATAAGGAGCACTGCATGG
CTTGGATCGTTTTCTGATCGTGTTGTTGTTCTGTTGTTTCTCGATCTAGATGTTTAAGT

TATTGGATAGTTTCTGTTTGGCACATTTCGTAGGTGTTTGTTTTTTCTAGTTAATTATA
GAATTGTGCTGTGCTGTGCTGTGGTGGTTTTGTTGATGTTGGTGTTGTCAGCTGCAAT
T

CAGTTGATGACTTGTTGTGTTTGTTCAATTTAAGTAGTTTATTGTTGTTTATGAATTG
AGGTGACTTTGGAAAATGAAATCTTTTCCTCAATGTTAAGAGGAAAACTAGGATAAT
GAAACAGAAATTTATTTTAAAATGATTG

>PUT-187a-Lens_culinaris-1105 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

CTGAAAGAAACTGTCCAATTGGAGACTGGAAGTGAAGAGGAGCAATTTTATTGTTG
TTGTGAGAAGAGTCTGGAGAGGAGGAGGAGGATGTTGCTGCTCTAACTACAAATCC
TCTTGTTGTTGTTGTTGTTGTGGTTGAGATTCTTCTCCCATGGGTATGAACTTGTCTG
GAAGTGCGTAGCTTAGCAGTAGTAGAAGAAGGAAAGTGAAGAAGGCGAGATCCGTT
GGAAGGTGTTGGAACGAAGATTGAGACGGCGGCGCCTGCAACCTCCATAATTGAAT
TGAATGAATGCAAGGAAGGAAGGGTCTAGAT

>PUT-187a-Lens_culinaris-1231 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

CACCATTCACCATGTCGTCATCAAAATCAAAAGCTTTATTTTTAATCTTCAAGATATT
AATGTTGAATATCATAACACCAGTAATGATTCATGCATGTGGTACATGCACACCAAA
TCCACCACCTTACCACCACCACCACCGGCCAAGCCATCCAAAACATCCACCGCATCA
CGGCGGAGGAAAAGGACGTCCAATAGTGACTCCTCCTCCAGTTGTTGTCGTGCCACC
AATAATCGTCACTCCACCACTGCTACCACCTCCGACTGTCATATACCCTCCACCAAC
AGTCTCTCCTGTTATTCCGCCACCAGTAGTTCAACCAACTTGCCCAATTGATGCACTC
AAACTTGGAGTTTGTTTGGATGTTCTTGGAGGTCTTGTTCATGTTGGAATAGGAAAC
CCTGTGGAGAATGTGTGTTGTCCTGTTATTCATGGATTGGTTGATCTCGAAGCTGCTA
TTTGTCTTTGTACTGTTATTAGGGCTAAGGTTCTTAATCTGAATATTTTCCTTCCTCTT
GCTCTTCAAGTTCTAATCACTTGTGGGAAAACTCCTCCTCCTGGTTTTGTTTGTCCAC
CTCTCTAAACTATAAGTAAAGCTCTACATGCATGCATGCTGCATGCATTATCCATAT
ATACTTAGTATTATCAAGCTAATTAGTAGTTTAGTTAATGTCTAGTTATTTGCTTTCT
AAATTATGCGTTTTTACCTTCTAATTAGGATTTTCGGGTTGATATGTAATGTGTGTAA
GTACTATATTATGCATTAGTGCATTATCTCCTTTTATCTATTTGTCTTTTTAT

>PUT-187a-Lens_culinaris-1263 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

GACATGAAACAGAGTGTGCTTTTGTGATGTTGGCGGGTTTGGTGGTTTGTGTTTACCT
TCACAGAGGATGCATTCGAAAGGAGAACAAGGGAACCAGACAAGGTCGCTGCCTGT
GTCCATATAGAGAGTTATGAGTTGTGGAGGGTGTGAACCTAGGTTGAAGGAAAGTG
TGTAATCACTACCGGGAGAAAGTGGGAGAGAAACTTGGGTTTGGTGGTGGTGGTGG
TTGTGTTGGTGGTTGAATCGTTTGGTGGAACGGGTTGAGGTTGATTTGAGGAGGTGG
TGGGTAGTGTTGAATTGGGTTTTAGAAATTGAGTGTGTTAAGGGTAATAGAATCATT

TGAGATGAGGGTGAAAAACAGAGCATGAAACAGAGTAGAAGTAGCAACAACATAG
GAGAAGACATTGATTGTTTGTTACTAAGAGGAAGAGAGAAGA

>PUT-187a-Lens_culinaris-1271 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

AGGAGAGAAAGAGACGACAGGAGACAACAAATAGAGAGAAAAAGAAAGAGAAGA
GAGTGAAGAGAAAGAGAGAGAGATGAAAAGAAACTGTGGAAACTGGCTTTCAA
AACCGCAGAAGAGAAAACGATGTCTGAGAGCTTTACATCATCATCCTTTCTTACCCT
GCAACTCTAACAGGACTAAGGAGGTGGTGGTTGCGGAGAAAGGAAGATGATTTCAA
CATGGGAATAGCAGCAGCGCCAGTACCGGTTTCTTCTTCGTCTTCGGTTGTTTACTTA
AAGAAACACCATACCAAACCTTGAAATATTGTTCAAATCCTATGTCACAAGATTATC
AGCAAGCAATCTTTGGATTCTCCTCGAATGGATTCGAGAGATCGTCTCAGCAGCAGC
AGCAG

>PUT-187a-Lens_culinaris-2033 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

TTAAAAGAGCACGGTTTGCCTCAAACAATGTTTCAAGTTACACGTATTTCCCACTGT
ATTTATATACATGACTAAACAGATAAGGAAGACGCCAATTAAGATCTTCAAAGACA
ATTTCCATAGATATTTCTCCTGAAATAAAACCACCCAAAGTTTATCAATAATTAAAA
CCTCTTCAAATCTTGCAGAATCCAACTCCTGTAAATTAAACTATCATCAAAACGATA
ATGGCTTCTTCAGAATCTCACACCACCGCAGGTAGCGGAGAAGGGAAGAAGCATTC
AACGAGCGAGCTTATGGCAAGCGCAAAATTGGTAGCAGAGGCAGCACAATCAGGTT
TCGGACCAGGAGCTGATGGGAAGGCGCTAGACAAAGGGAAAGTGGCTGATGCAGC
AGGAGATCTTCTAGATGCAATTGGTGATTATGCTAAATTGGATGACCAGAAAGGGTT
AGGACAATATGTTGATAAGGCTGCTGATTATCTTCATCATTACCACCCCACCACCAC
CACCACCACCACCACTACCGGTCATCATCCAACTTCCAAACCAGATCACCACAAAAT
CGATGATGCTGCCAAAACTGACGGTGGAGAATCAGGTGGACATGGCCATGGACTTG
GTGATTTTGCGAAGGCTGCCGGAGGTTTGTTTCATAAATGAAAAGAAAGATCATTGA
TTTCGACTCACACAAACAGGGCAGAATATGTTTGAACTAAGATCCTGTGCCCTGTTT
GTCTATGTTTTTAACTAATTCTGTTGTATGTAATGATTCAATCCTGTTTATTTTCTTTG
TATTTAATTTAGTCTCTTTTGGCTACATGTAATTTGTTCATGTCTATGGTGAAACTTTG
TTCTCTTATCGTGTTTTCTATTTTAGCAACACAACAACTTCATTAGACAAAAAAGAA

>PUT-187a-Lens_culinaris-2096 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

TAAGTAGAAATTGTCAGAAACTGTGGCCAAATTTGAATTCTGAACATTGAAATCTCT
CAATGATTCAAGAGACTTGTACAATGAAGCAGCCACTCTTTCTCCTTTATTGCATACT
ATTTTAACATAAAACCCTCTTTCATCTACTTGAATCATATCCATCTGGACAATTTTCT
TGCATATTGAACTAGTGTTATCATTGAATTGAATCTTTTTGTGGCTTTCAATTGATCC
TTGATGAGTTTTAGACACTGCTAAGGATGCTTCAAGTCCAGAAACCTCAGCCTTAAG
CTTCTTGGCTTGTGATTGAAGTTCTTGCATGTATGAAACCGCATCTCCAATTATAGAA
GCCTTATCCATCTTAGTTATATTGGGAACCAAAGATCGCAATGCGTAAAGCTTATCC
TTCATCCGACCTCGCCTCCTCCGCTCTGATTCCAATGTCTTAGACCTATCGTTTTTCA

ACCTTGGCTTAGTATCACCACTTGTAGTTGTTGTTGGAGAAGAATAATTATCATCATC
ATCATCATTATCATTTTCTTCTCTTATTTCCCCCTTAGCTTCTCCATCAAAACAAGAG
AAAGAGCCAAGTGAAGAAGTTGGATCAAAAGGGGTGACAAAACTATTATTGGAGTG
ATCAAATGTGTTTGTGGGAAATGAAACAAATGAGTTATCAACAAAGCTTTGATTGAT
TATTAGGTTAGAATTGAAGTCACACATAGTAGTATCTTCATTTTCTTCCCCACGAATC
AAGTTGATGAATTGATCAAAGTTTGGATCTTCAATGAAATCGTGTAACTCAAAGCCA
TTGTTAATGTGAGCAAGTGTCTCTGGATAAAAATCCATTGTGTGTATATTTTGGTTGA
AAGTTG

>PUT-187a-Lens_culinaris-2104 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

TCACCCCTCCTATGGAAACAGTTTTTTTGGCCACTCCGATGCAATTGCAGCCAGAGT
GGAATCAAGGTGGTTGTGTAGCTTCAACTTAACAACAACAACAACCCCTGCGTTTGT
AAACACACGTTTCCTTTGTCTGTGTCTGTAAATAATAAGACAAGAACGAGTTGCAGT
TGCAGAGGGAGAGACGATGATCCTCTGTCACTTTTTCTGCTTACGCCGTTCTGGGAG
TCCAACCCAACTGCTCCGGCGCTGAAATTAAAGCTGCTTTTCGAGCCAAAGTGAAGC
AGTTTCACCCAGACCTCAACAGAGATGAAAATGAAACATCGGATGTTATGATTCGCC
GCATAATTCAAGCATACCAGATACTATCCAACTACACACCATCACAAATTATTGAAA
CGGAGTGCTTAGATCCCTTTGATAGACCAGGGTGTGAAGTCTTTTGATCTTTTTGTTA
ATGAGCTTCTCTGTGTTGGAAAAGCTTGTTCAAATCCATGTGTTGAAAGAGCATCTC
ATGCTTTCACATTTGTCCCTTCTACTGGAACAGCACGTGCGTTTTCTCAAGATCAAGG
GGAAGATTACCAAGTTCAGTGTGCTGTTGGACAATGCCCTAGAAGTTGCATTCACTA
TGTAACCCCATCACAGAGAATTCTTTTGGAGGAGTTACTTCACAGTATACTGGAAGT
ACCATATGATACATCAGCTGAGGCAGAATTACTCTGTTCACTTATAACCAAAGCTAA
GTTTGAGAATAACCGATACCAAAAGCCAAAGAAGAAAACCAAATTTTCAACACAGC
ATGTTGATTGGTTTTAACGTCTCCGTGTCTTCACTCCCAACCTATAGATACCACTCAG
ATTGATGAGACAATCATGGTTCTCAACGCCACCAGTATAAAGTTTATCAGCAACTTT
TACTTATACCTATAGTTAGCAATGAGATTAGGC

>PUT-187a-Lens_culinaris-2213 PlantGDB-assembled Unique Transcript-fragment derived from Lens_culinaris mRNAs Jan_31_2012 (based on GenBank release 187).

CATCCGTCTCTCCTGCGTTCATCTCCTCTGAACGGACATCAAAACTACAGAAAGAAA
CTCAACCAAAACGGATGACGAATTTCTTCCCAGATTTCTCTCAAACAAGACCAAACA
AACTAGCCCTAATCCCAATCTCTTAACCTGAATCTAAAGTTATAAACAGAAACACAT
CTATCAAAAACTATAAATCGACCTTCAGAAAGCTTGATTCAAGCAAGTTTAAACAAC
AACAACAACAGTTAACTAAAATGCTAAGCAGAGTCATACATTATTCAATACGAAAG
AAAAGAAGTACCAAATCAGAGAAAAAAAAATTCTCAATGAAGAACTACAACGATCCA
GAAGACTAAAGGTAACATGAACTTCTTCCCTTTCAACCTGATCTTCTCCTTTTGCTTG
ATTTTTTTCTGGTGTAGTTGTCGTGATTTCTATATACTGTGTTGTTGTCTGCGTTGTT

Unigene sequence of the polymorphic marker UN0003.1

>UN0003

GAAGGATAGGGAAATTGAGGTTTTTAGAAACCAGGTTGAGGAATTGGAGAAGGTTG
CAGGTGAGAAGGAACACGAGTCGGGGGACTGGTCTGCAGAGAAACTGAGATTGCA
GAAGGCACTTAAAGAATCTGAGGAGAAAGCAAAGGGTTTCGAAGCGAATATTATTC
GATTGCGTGAGGAAGCAGTGGAAACTGAGAAGAAGATCAAAGCACTGAATGAAAA
AGCTGTTGAGATAGTCGATAGAGATTTAAATGGGATACAGCGTGAAAGGAATGAAG
TTAAGTTGCAGTGGCCGATTGTAGCGGCGGGAGCAGGATCTACCGTGGCTGTTTTTG
GGGCAGCAGCTTTGATCTATGTGTACTGCTCAAAACGGAGGTGATTTTTCTCTGATG
TGAGAGTAATCAGAGGAAGGGGAATTTGAATGGGATGTTGTAGAGGTTAGATTTAC
AAGTCTTTTACTCACAAATATGCTTCAATTTTGTTCGTCATAAAATAATGATGCTTCT
ATGGCTTTTGTTTTTGAAGCCTGTTAATTTTATTTATGTCGTAGTTGGCATACTGGTTT
GGTTGTAGTTGCTTGTTTTATGAGGATTAAAACCATTGTGTGTTTGGAGCAATGCTCT
GATTAACTGCAGTTACTAGGTTTTTGGTGTACTAGTGATTACAAGTCTATTGTTATAG
TTGCTTTGGAAGTTTGGATTTACAAAAAAAAAAAAGTTAAAGCTTCATCATCTGAGA
AAAAGAGAGAAAGCTACATTGCAAGAAACATGCACAATCCACCTCCACCAGGTCCA
GGAGGTCCAGGTCCTCATCACCACCCTGGGCCACCGGATCCTCATCCGCCACCGCCA
CATGTTCATCCACCTCCTCCAGGACCAGCACCTCCACCTGGTCCACAGCCACCTCCA
CCTGGTCCTCCCGGTCCTGGACCACATGGCCACCCTCCACACCACCACCACCATTGA
TTATATGAATATGTGATTAATTAAGATGCTGCTAAGCTTTTATTATATATTGAATAAT
AACTTAGGTTGATCTATATGGGGTATTTATTGTACTGCAGTGTTGAATTCATTCAACT
ACTTATGTTTACCATGTATCACTGTGTGGTTATTATATTATGCTTTGTGTGGCATGTA
TATGTAAATTGCTAGAAATAAATAAAAAGATAAGTGGTCTCGAGTTTTTTTTTTTTT
TTTTTTTTTTAAATCAAAAACACCAAAGTTGAATTTATAACACTTCAAAAAACCAGT
ATTTGTTCTTCAAAACAAACTCCTTTCAGAACAATTCATATTATTTTCAACAACCTAC
CCCTTCACTTCAATTTGAAAGGGTCAAGGCAAAGATAACAAGAGAAACCAAATTCT
GGAAACATCCGGTGAAAGAAGTAAGGAGAAGGTAAAGGTCGCAATGATGTTAAGTT
TAACATGAAACCCAAATTTGAAAGGGACACTTTGATTCACTTAACTAGACATGGGGT
GGTTAGAAATGTACTCAATAGCAAGCGGACAAGAGTCAATTTTATCAGCTTCCTTGT
CTGGGATTTCAAGCTTGAACTCTTCTTCTAGTGCCATTACAATTTCCACGGTGTCTAA
GCTGTCCAAACCCAAATCCTTCTGAAAATGCACTTCTGGAGTCACCTTAGAAGGATC
CACTTTGGGGAAATCTTTGATGACAGAGAGTACTCGATCGATGAC

Unigene sequence of the polymorphic marker UN0032

>UN0032

AAGATTTATCACTTATTTTTGATACTGGTAGTGATCTCACTTGGACTCAGTGTCAACC
TTGTGCTCGTTCTTGTTACAAACAAGTAGATGAAATATTTGATCCATCAAAGTCTAG
TTCTTATTCCAATATCACATGTACTTCTCCAGATTGCACTCAACTCTCTTCAGCTACA
GGAAATGACCCGGCTTGTGCTTCATCAACAAAGGCATGTATATATGGGATTCAGTAT
GGCGATCAATCTTTTTCGGTCGGATACTTTAGCCGCGAACGGTTGAGTGTAACGTCG
ACCGATGCAGTCGACGGTTTTTTATTTGGCTGTGGACAAAACAACCAAGGCCTATTC
GGCGGATCAGCCGGTCTGTTAGGCCTCGGTCGTCATCCAATCTCGTTTGTCCAACAA
ACCTCTCAGAAATACTATAAAAAGTTTTCTTATTGTCTTCCCTCCACTACTAGCGCCG
TTGGCCACCTCACATTCGGTTCCACCAATAGCAAATATGTAAAATACACTCCTTTCTC

CACCGTCTCCCGTAGCGATTCCTTCTACGGACTCGACATTTCTGGGATCAGCGTCGG
CGGGACAAAACTTCCGATCTCATCCTCTCTTTTCTCCTCCAGTGGCGCCATTATTGAC
TCAGGCACTGTCATTACACGGTTGCCACCTACCGTCTACACCAGCCTCCGCGATTAC
TTCCGCAAAGGCATGGCGAAGTATCCGTCTGCTTCAGCTCTTTCAATATTGGACACA
TGCTATGATCTTAGCGGGACCAAGATTGTGTCTGTTCCAAAGGTTAATTTTTTCCTCG
AAGGCGGTAACACGGTGGAAATCGCCGCGCCAGGGATACTTTACGTGGCGAGTTTG
AAGCAAGTTTGTTTGGCTTTTGCACCAAATGGTGATGATAGTGATATTACTATCTTTG
GGAATGTGCAACAAAGGACACTAGAGGTTGTTTATGATGTTGGTGCTGGTAAGATA
GGTTTTGGATACAATGGTTGCAAGTGAAAGTGAAGTCAGTTTTGAATATTTTGATCA
ATGAAGTATTCTTATTTGAACACACACATATATATATATCCAACAAACAAAATGAGA
AATACATAAAAATAAATTGTATTTTGATTTTTGGATTGGTCATGGTTATAATTAAAGT
GTTTCTATAAGAATTTCTCTTGATCAATTTATATGATTATACATATGCTTTGGGCTGG
TTAGGGAGAAAGAAATCTTAATCGGCCATGATATTACTTACTTATTAATGTTATTGC
ATACTAGCACTAAATAATTATGTGGG

Unigene sequence of the polymorphic marker UN0033

>UN0033

CCCAAGCCAACCATTTTTGCATCTGTTAGAAACACCAACAAAAAACAGACCAAAAA
AAAAACAATGGCTGTCTCATCTTCTTGTTCTCTTTTGCTTCTCTTTCTTGCCTTTGCTT
TTGCTGCCGCAGCACCAAAGGATTACTTACTTGGTGGCAAACCTGATGCATGGAAGG
TTCCGTCTTCTGAATATGACTCTCTCAACAAATGGGCTGGAACTGTCCGTTTCAACAT
TGGAGATCATCTTATTTTTACCTATGAAGCTGGAAAAGATTCTGTTTTTCAAGTGAGC
AAAGAGGATTACTTGAGCTGCAACACATCAAACCCAATCAAGAAGTACAATGATGG
GAAAACAAAGGTTAGATTTGACCGTTCTGGTCCATACTACTACATCAGTGGTGAGAA
GGGTCACTGTGAAAAGGGTGAGAAGGTTACTGTGGTGGTTATAAGCCTAAGAAGTC
CAAGTGTTGTTCCTGTTTCACCTGTTTCTGCACCTTCACCTTCACCAGCTGTTGCTCC
AAGTCCAACTAGTGGCGCAGATGTTTTGAAAGGTGGTGGTGTTTTGATGGCTATGGG
AGTGGTGGCTGCTATGTGGATTTTCTAAATTAAATTATCATCTTTTTAGTATTTATAT
TTTTATTATTATTATTATTATTATTAATATTGGGATTGGTTTAAGTTTGTTAGAGACA
GTCACAGTGAGGTGATTGCTAGTATAGTCTTGGTATTTTGTTGGGACCATATTGTTCA
ATTTTTAATATAAATTATGAATCAAATTATATGGTTTGGCCTTCTTTTTTT

Unigene sequence of the polymorphic marker UN0046

>UN0046

CTTCTTCTCAAATCCAATGCTTTGTACTTCAACTCGCATCCTCTTCACAACTACAAGA
CTAGCTTTAGTTTCTTCTTCTTCTTCCTTTCACTTTTTCTCCACTAATTATCCAATC
TCAGTTTCTTCTCTTCCAAACAAATCTTTTACTAAACCTAAACCAATTTTTACGACTC
CAACCCCTTTGTTCTTCACTCTAAGAACAAATCACGCCATGGCTTCCCCATCAAACC
CCCAATCAATTTACGATTTCACCGTTAAGGATGCTAAGGGTAATGATGTTAATCTTG
GTGACTACAAGGGAAAGGTCCTTATCATTGTCAATGTTGCTTCACAATGTGGTTTGA
CTAACTCCAATTACACAGAGCTGAGTCAGTTGTATGAGAAATACAAACAGAAAGGT
TTGGAAATTCTGGCATTCCCATGCAATCAGTTTGGGGCGCAGGAGCCTGGATCTGTT

GAAGAGATACAAGACTTTGTGTGCACTCGCTTCAAAGCTGAGTTTCCTGTTTTTGAC
AAGGTTGATGTGAATGGTGCCAATGCTGCTCCAATCTACAAGTATCTAAAGTCAAGC
AAAGGTGGGCTCTTTGGGGACGGTATCAAATGGAACTTCTCCAAGTTCCTTGTTGAT
AAAAATGGCAATGTTGTAGAACGTTATGCACCCACAACATCACCTCTTAGCATTGAG
AAGGACTTGTTGAAGTTGCTTGATGCATGAGGAAGAGTTATGAATGTTGGAACCTGG
AATAAATAAACATGGATGAAGAGAACTTTACTATTTTGTATGTGAATAAAGGAATGT
ATTGCAGGCACATGCTGGTGCAATTCCCTCGATTTGGTATACTTACCCAAACAGTTG
TCTCGTATCTTTGGTTTTGGTTCTTCTTCATGAGACTGTGTACTTGACTTATTACCATT
TTATGAATGTAAATAAGTCCCGTT

Unigene sequence of the polymorphic marker UN3776

>UN3776

CATCTTCATTGCGATCGATCCTTTCCCATGGTTTAGCAACTTCCTTCAAACCTCAAAT
TCATCCTCACCGTCGATCTCACTCTATAGAACCTTCTCGAAACTGAACCAAATCGCG
TAGATCTCAAATTAGGTTTTATGGAAATGGCGTCAAGTCCTCGCTCTGTTGAAGAGA
TCTTCAAAGATTTCAGTGCTCGTAGAGACGGAATCGTTCGTGCTTTAACTCAAGATG
TTGATGAATTCTACACTCTCTGCGATCCAGGTAAACGAGAAGTTGAAGATCTGAATT
TTTTTGTTGTATTTTTTTCGAATTTAATTGTTTCTTTGTGAATTGTTAAGTTGTTACTT
GTTATAAGAGTTGTGAGAGTTGAAGCTTTTTATTTTCGGAGAATTTGAAGAAAAAAT
AAGTCAAAGTTTAACATGTCCCATGTGTTTGTTTTTTTTTACTATTTATTTGTTTGTTT
GTTAGTTTATAATATTTATTTATTTATTTATTATATTGTTTATTTATTTATTTGGGCAC
GAATTCACACACTCAACTAGTTCAACTATAAAACAGGTTATCAGTTAATAAATTCAG
AACTATGAAGCACCAATCCCAGAAACATGACAGCGATACCAGAAACATGACACTGA
CACGACACAATGATACTAGTAATAATTTGAAAGTTGAATAAATTAAACATTGAAAT
ATTTTTTTTTAGA

Unigene sequenec of the polymorphic marker UN3302

>UN3302

CCTAATCCCAATTCTCAATGGCACCACCAAAGAGACTCCGCCCTGCTCCGCTCGATG
ACCCACCCTCCGCCTCCTCCTCCTCCTCCGATGACTTCCCACCGCCTGTATACAAAAA
GCCAGAAGATGAAGTTGTCGACGAAGAAGAAGATTCATCCGAAGAAGAAAATGAC
CAGGAAGAAGACGATGAAGGTTCTTCTTCCGAAGAAGAAGAAGTTCAACCTCCATC
CAAAAACCCTCCACCTTCTACCCCAATCTCGAACCCCAACCCTAAATCCGAATCTGG
TTCCGAATCCGAATCCGGTTCTGAATCCGGATCAGAATCCGGATCCGAATCTGATTC
TGAACAACAACCTACTCCACCTCCCAACGCCAAAGTTAAGCCTCTCGCATCAAAGCC
CATGAAAGCTCAACCACAAGCCCAAGCCCAAGCACAATCCACTCCTCTACCGGCCA
GATCTGGTACAAAGCGTGCAAACGAAAACGGTTCTAAACGTGCTACGAAGAAAACA
ATCACTGCCAGTGGTGGTTCCCACGACGAGAACGATGTGGATGCAGACGGAGACGT
GAAAATGACCAGCGAAGACTCTAAGAAGATGTCTCAGAGGGTTTTCACCGAAGTAG
ACGAGATCGCCATTCTCAAAGGTCTCGCCGAGTTCATGTCAAAAACTGGAAAGGAT
CCAATGAAGGACCCGGCCGCGTTTCATAGTTTTGTGAAGAAATCGATTAAAGCTGAT

GCTAACAGCGAGCAGCTGAAGCGGAAGGTTCGTGGTTTGAAGTTGAAGTTCACAGG
CGGTG

Unigene sequence of the polymorphic marker UN3176

>UN3176

GTTTGATTAAACCCAGCCCAAATGACAAACCCCTTGTTCGGCGGCTCCTTCGTACCA
CCCTGACTGAGAACACGAAATGGCGGTGTCGTCGAACTGTGTTCGGAAATCTCTCCA
AATAGCTTCATCTTCGGCCAAAACCCTATTATCTCGTCGATCTCCTTTACCTTCTTCTT
CTTCTGCAAACCCCAACAAATTCAACGCCCCTGCTTCTTCTTTTCAAGCTTCCCCACA
CAAACGCTCACTCTCCAACTCCTGGCTCCCGGTGCAATTAGCTGGTGCACAAGTATC
ATTGACGCCATTGCATAGTGCTACTGCTTCTGCATTGTTCACTTCACTCTTGTCTTTG
CATAATAACAAATGGGGTTGTCTTTCAGAAGGTTTTGCAACGACTTTATAACAGCGA
TGATATCTTTGCTTTTAGGCCGCCAAGATAGTGTTATATGCAGGTTTTCTTCCAACAC
TTGTTTCTAGTTGGTGCTGCCATTAATAATGTAATTTGATTTTTTTTTTCTTTATTTAC
TGAGACTCTTGGGTCATTACAAGGTGTTTTCCCATTTGAACTTTCCATTTTTTCCTGA
TGGTTAACCCTTCATTCTGGGAAATTTTTCATGTAATATGTATACCAGGTTATCAAGT
GATTTTATTT

Unigene sequence of the polymorphic marker UN3814.1 and UN3814.2

>UN3814

CTTCTTCATATCTCGGTAGCTGCTAGTGTCACTGCTAGTGGTTACGCAAAAAAAAAA
ACTTCTTTTTTCAGGGTTTGGGTTCTTGGGTAGGATTCTTGAATCGAATTGATTCGTT
TTTGTTTCTGGGTGTGATTTGTTTTTGCAATATTAATAACAAGATTAAAATTTAAAGA
AAAAAACAATAACAAGATGGTTATTTCCTCTGATGAGAACAAATCGAACGCAGTCA
TGCCCAGGAATTGTCAAGGTGGTGGTGGAAGAAATGCTGGGCAGAATAGAAGAACT
TTGAGTGTGATCAATCAGAATCTTGTGCAGGGTCGACCTTACCCTTGTGTTGTTAAC
AAGAGGGCGTTGGTTTCTGAGTAATTCTCTTTTTCTGATTTATTGTTGTGGATTTTTGT
GATTCTTGTTTTTTTTTTTTTATTTCTTTAAGGTTGATTGTCTTGTTTTCTTTTTTGTGT
GTTTTTACAGCAAACATGAGATTTGTGAAAAGAAACAGGCTGATCTGGGACATCGA
CCCATCACAAGGAGATTCGCTGCAAAAATTGCCGGTTCACAGCAGCAATCTCATGCT
GAGAAACCTAAGAATTCAAATCCATTGAATTTGAAGTCCAATGTGTTTGGAAAGGCC
ATAGCTGTTGATGATGAACACAAGACACCGGCAGACCAACCCGTGCCTATGACTTTA
GAGCAAACTGTACCAATGCACGGTTATCCAGATGAGATGGAA

Unigene sequence of the polymorphic marker UN3720

>UN3720

GAAATACTTCTCACTCACCCGAGAAACTCAAACCTAATCCACTTTCTTTTTAATTGAA
GTGGTCGATCATCATCACTAATAAGAAATGGAAAACATTTTCATTTTAACAGTTAGT
TAGTATTACTACAAATAAGAAAAAAAAAACAGACATTGTTACCCCTTTTTGTATCAG
AGTCGGACCAAACTCAACTCGCCGGCAGCGCATCAAAGCATTGCGTCGCAGAAGAT
CATAGCTCCACTTCGCATCGCCATTCCTCAATTAGGGTTTTGATGTTTGCATAATTTC

AAGGATAATTTCACAATGAGGCATAGTCATGGTCCATATCCTCCTCAACTACTGGAA
AACAAACTCGCTCATCAAGAAGCAGAAATCGAGAGAATCGCCGGCGACAATCACAG
ATTATCAATCACACATAGAGCATTAAGAGATGCACTTGTTGATGCTGCACAAGATGT
GCAAAAGATAAAATCACACGTTAGAAGCACTCAAACTGAGAGTGATATTCAGATTA
GGGTTTTGCTTGATAAGATGGCGAAAATGGAGGTTGAGGTTAGAGCTGGCGATGTT
GTCAAGAAGGAGCTTCAACAGGCTCATATGGAGGCGCAGAATTTGGCTGCTTCTAG
GCAGGAATTGAGGGCTCAAATTCAATTGGCCTCTCAGGAATTGAAGATGGTTGTTGG
TGATCTTAAAAGTATACCTGATTTGCATGCTGAATTTGATGGTTTAATGCAAGAGCA
CATGATAATACGTGACACATTCGACTATGAAAAGAGT

Unigene sequence of the polymorphic marker UN3519

>UN3519

GAACTTCAACTTTCTTTACATAATTAAAAAAATATGTAAAATAATAAATAACGCAAA
CCAAAAAAAGAAAAGAAATAACCTAAAGAAAACAGCTGTGTGATGATTTGCTGTAA
CCTCATATGAAGCAAAGAATAAAGAATTGGATTCTTTCTTCTTTCTTTCTTTCTTTTCT
CTTTCTTTCTTCTTTCCTTTCTCTCCCTTTTCTTCTTGACCGAGATTCTTTCCTTTTTTTT
CTTTTTTTTTTCCGTATTGTATTTTTACATCCAACTTAATTAAAAATCCTAACAAACTA
AAAAGATATTTCAAAAATAAAAAAAAAACAAATTAATTTACAGGTTTTCCATATCCG
TAATACGAAAGATACCATTTCACGAATTTCTTCAACCCGGTTTGTAAGTCGGTTGTG
GGTTTATACCCGAGTTCTCTTCGGGCCGAACTAATATTCGCATGCGTAAACGGAACG
TCGCCGTTTCCAGGCATGTCCAGAATATTCCTCTTCGCCTTCACCTTCAAATGCCGTT
CTAGAATACTCACCAACGTCGGAACCGTCACCGGAGAAGTATTTCCGAGGTTGAAT
ATCCGGTAAGGTGCGGGTCCTCGTTTCTTGCCACCGGATCCGGTGCTCTTCCCGGAA
GTATCCAACGATCCGATACATCCTTTTACAATATCGTCAATGTATGTGAAATCACGA
GCCAGATCGACGTGATTCTTGCCGCGATAAACCGTGATAGGTTTCCCCTGGAGAATG
TTCCGGGTGAAGGA

Unigene sequence of the polymorphic marker UN3311

>UN3311

TTTCTCCCTCAACCCCTTTTGTTGCTCTCTAACCAATCTATTACACAAATTTCCATTTA
TCTTTCTCCATAATTAGAAGGGAGAAGCTTGTGTCAAACGTGGAACGGTTCTGAAAT
TACTCTCTAGGGCATCATCATATTATATATTTCAGTGTTTTTATGTCATAACTTGCAT
AACCTATGTACGAGTGTTGAAGACATGCCTGTGGTGGTTGATATAAGCTCTGATGAA
GAAGAAGATTTGAAAGAGGGATCCGAAAATACTGATTTGGAATGGATTAAAGAGTT
GCTCTTTAATTCTGGAAATGAATCTGATTCTGACGGTGATTCTGATGTTGTCTTCCTT
CATGAGAACAAACCACATGAGATGAAATCAAAATCTTCAACTTTGCCTGTGAAAGTT
GTTGACGATGATGATGATGATGATGAATGTGTAGTTCTCGAAAATGACCCTGAAAAT
GGTGTCACTTCTGTGGATGATGAGGATGCAAATGGGTCAGATGAGTTGGTTGTGGTT
GGAGAGAAGGGACAGGTTGCATGTAGAGACTATCCTCATGCCCGACATCTTTGTGCT
AAATTTCCTTTCTCTTCCACCCCTCATGAGAAACATTGT

Unigene sequence of the polymorphic marker UN3728

>UN3728

CACTTTTCTTCTCTATTGAGCAAAGTAACATTCATTACATACATACACATATAGATAT
AGTTTCTACAAACATTGAACTTTCTGAAGAAAATCACTGAAGAAGAAGAAGATGGA
GATCAACAACAACAATCAACAATCGTTTTGGCAATTCAGTGATCAACTTCGTGTTCA
CACATCAAATCTTGCAAACTTATCGTTAAACGATTCAATTTGGGGAAACAATTACTC
GTCCACCAAAAATGAACGAAGAAATTTCGATATCAAAGTTGGTGGTGAAATCAACA
ACAACAACAAAAGTGATGGTTGGAAACAGATGAACAACAACATGGTTGATGTTGGG
ATTAACGGTGGTTTCAACAAAGGAGTTTATCCAAATTCAAACTCTTCTTCTTATGGTA
ATTTTAATAGTAACAACAACAACTTGAATATCAATTTCAAGGGAGTTAAGTTTGGTG
GTGCTAAGGTTGAAGATGAGAGTTTTCATCTGGCTAAATCTTCTAAGAAGAACACAA
ACCTTAACAAAAAACATGGAGACAACAACAATAGTAACAGTGATGGGAACAAGAA
TAAGGATGTTAAAGCTGCTTCTGACAAGAGATTCAAAACGTTGCCACCGACAGAGT
CTTTACCTAGGAATGAAACGATTGGTGGTTATATCTTTGTTTGTAACAATGATACCAT
GGCTGAGAATCTCAAAAGACAACTCTTTGGTATGTTCCTTTACTTTCCTTTTTCTTCTT
CAATTATGCAATTGTTATTATTATCTCTGATCTTTGATGTACTTTTATTT

Unigene sequence of the polymorphic marker UN3652

>UN3652

ATTACTATTACCTCTCTTAACTCAACAACTAGTCTTTCATTTCTTCTTCGTACTAACA
ACAACTATGAACAAGAACAAGTTCCCTTTTACTCCTTCGCAATGGCAAGAGCTTGAG
CATCAAGCTCTTGTTTACAAGTACATGGCTAGTGGTATCTCTATTCCACCTGATCTTC
TTTTCACCATCAGAAAAAGCTTTTTGGACTCTCCTCTCTCTTCAAGACTTTTTCCTAA
CAACCAACAACATCACTTTGGATGGAACTATCTGCAGATGGGTTTGGGAAGAAAGA
TAGATCCTGAGCCAGGTAGATGCAGAAGAACAGATGGGAAAAAATGGAGATGCTCA
AAAGAAGCATATCCAGATTCTAAATACTGTGAGAGACATATGCATAGAGGAAAAAA
CCGTTCAAGAAAGCCTGTGGAAGTTCTGAAAACAACAACAACAACTAATAATGCTT
CAACATTTGCAAACTCATCAATCACCAAAAGTAGTTCTTCTTTGTCTTTTGATACACA
ACAACACCAAAACTACCCTCAAAATTCTTGCTATGGTTCCAATTTGCAACATTCCTTT
GTGTATCCTCATACTACTTCAAGGTCATCAGCATCATCTGGAATTGGTTTGTCATTTG
AAGACAATAATGGTTCCTTGTTTCTTGACAGTAACTCTTGCTCTCAGAATAATGGAG
ATTACAGGTATGTGTATGGACAAAAAGAAGAGGTAGATGAGTATGCATTTTTCAAA
GAACCTTCTGGTAGCACTACTATG

Unigene sequence of the polymorphic marker

>UN3321

ACCAAATCCCTATTTTCCCAACTCTAGGTCACAGCTTTTGATTATTCTCTTAATCCAA
TTCGTCATCCAATTCCCAAGTGTTGGATTCTGAATGCTTTGATTCCGAACCTAGCGTT
CGGATTCATTTCTACAATTTTAACCTTATAACAGGGTGGGTATGTTTCATGCAAAGA
AGTTTCCGGAGGGAAATATGATGCCTTATAAATCTCAAGGTGGAGGTGAAGAACTT
GGGAATGTTGGGGTTTTGAGTGGATCTGTTGTGAAAGATGCTGCACCTGCTGGGGGA
GGCGGGAAGCAGCGTTTGCGGTGGACGTCGGATCTTCATGACCGATTTGTGGATGCT

ATTACGCAACTTGGTGGACCAGATAGAGCAACACCAAAAGGAGTTCTTAGAGTGAT
GGGGGTGCCTGGTTTGACCATTTATCATGTTAAAAGCCATTTACAGAAGTATCGCCT
GGCGAAGTACTTGCCCGAATCACCAGCTGATGGTAAAGATTCTAAGGATGAGAAAA
GGAATTCTGGAGACAGCATTTCTGGCGCTGATTCTTCCCCGGGATTGCAAATCAATG
ACGCACTACGGATGCAGATGGAGGTTCAAAAACGTTTGCATGAACAGCTTGAGGTT

Unigene sequence of the polymorphic marker UN3548

>UN3548

CGGGATTTACCCAGGAAGAGGCATCAGATTTATTCGTAGTGATTCACAGGTTTTTCT
ATTTGTGAACTCGAAATGTAAGAGGTATTTCCACAACCGTTTGAAGCCTTCAAAGCT
CACATGGACTGCCATGTATAGAAAGCAACATAAGAAGGACATTGCCCAAGAAACTG
CGAAGAAGAGACGCCGTGCTACCAAGAAGCCATACTCTAGGTCCATTGTCGGTGCT
ACTCTGGAAGTCATTCAGAAGAAAAGAGCTGAGAAGCCTGAAGTTCGAGATGCAGC
TAGGGAAGCTGCTCTGCGTGAAATCAAAGAGAGGATAAAGAAAACAAAGGATGAG
AAGAAAGCCAAGAAAGCAGAAGTAGCATCTAAGGCACAAAAATCCGGCAAAGGTA
ATGTTCAGAAAGGAGGTTTACCCAAGGGTCCTAAATTGGGCGGCGGCGGTGGCAAA
CGTTAAGTAGAACGATAATTAGTTGCAATTTTGGCTGTAATTTTTAATACATTTGTTT
TTAAACTTCTAAATTGATATTTTATTTGCTTGATTAGCTGACCTTATTATCACTTCTGT
TTTGAGCTTCAGACAGTACCTTATTACCGAGTGCAAAAAAAAAAAAAAAAAAACTC
GAGACTAGTTCTCCTCGTTCTTCAAAGAACATAACAGAAGATGTTTGAGAACCTTAT
TCAATTTGTGAAGGAGCGCAACTTGGCTCGGTTCTGCTTAATGTGAAGCTTTCATGTT
GGATTGTTATAATCGCAGCGTATAACCTGCA

Unigene sequence of the polymorphic marker UN3414

>UN3414

CTCCTTCCATTTCTCTTTCTGCAATACTAATAAAATTTTCATTTTTCTTCTTCTTCTTCT
TCCATTTCTGGGGTTTCATTCATCATAGGATAGGAACATGTTTTCTAGGTTGTTACGT
CCTCACGACCATGAAGGAAGTAGTGTTGGTGTCGTTCAGGAAGACACAAATCATCA
TCACAATCACCTTGCTGACCCTTGTCTTGTTCTCACTTCTGATCCAAAACCTCGTCTT
CGTTGGACTACTGACCTTCACCAACGATTTGTTGATGCTGTTACTCAACTCGGTGGA
CCAAGCACCACACCGAAAGCGATCATGAGGACCATGAATGTCAAGGGTTTAACACT
CTATCACTTAAAGAGTCATCTTCAGAAGTACAGACTCGGTAAACAAGCTGGAAAAG
ATTCTGACGAGGGATGCAAAGATGGTTCATATCTTCTAGAAAGCCCTGGTACTGAAA
ACTCATCTCCTAAATTACTAGCTTCTGATGCAAACGAGGGTCAAGAAGTCAAGGAG
GCTTTAAGAGCCCAGATGGAAGTGCAAAGTAAGCTACATTTACTAGTGGAGGCAGA
GAAGCACCTGCTAATTCGCCAAGATGCAGAACGGAGATACATGGCCATGCTTGAGA
GAGCTTGTAAGATGTTAGCTGATCAATTTATTGGTGACACAGCTATAGACACAGACA
TCCAAAAATTTCAAGAACTACCAAGTACCGAGCTAGGTGGAATGCATGTTTCA

Unigene sequence of the polymorphic marker UN3326

>UN3326

GGCATCTTTTAGTCCATGTCCCCAAATGTCGTATAACATATTAACATTACAGCTCCCC
CGGAGTTTCATGCGCCAAGTTCTTTCAAAAAAAAAACATAGTCGTTCTTTCAAAAGA
ACAACATAGTCAACCGATGTATAATATATATTCCAAAAGATAATGAAATCCATTATA
GAAAGTTTGACTCAACTTTCCTACAATGTTACATTTGACGGGGCCCCAAAAAAATTT
GCCCCCCTGTATGAATTAACAAAAATCAATTTACAAGAAAGTTAAATTTCAAACAAA
AGTCCTACTAAATTAAGTCGTCTAGAAAATCTGAAGACTGGATAGTTCTACAAAGTT
CCCTCAAATTAGCCAATACATGGGGGGGTCCCAAACTCTTGGCACCAATGAAATTCA
AAAGCTGCTCTAAATCCTTGCTTAGTAAGAATACAGCATAAGTTGCTCTTGTTGTGT
CTTGCCCTTCTAAAAATAGGGGGAATTCTACATGCTTGGCATTTGTGGAA

Unigene sequence of the polymorphic marker UN3849

>UN3849

GACGACTTCAGTTGAAACAGCTTCAAGTTAGAGAGAGAGAGAGTGAGTGAAGGTTG
AGAAGAGAATGGCAAGAACATCTTCCACCACGAAGGATGCACAAGATCTATTCCGC
GCGGTTTGGTCTGCATATTCCGCCACACCTACAAATCTCAAGATCATCGATCTCTAT
GTTTCATTCGCTGTTTTCACCGCTCTCCTTCAGGTAGTTTACATGGCTCTGGTTGGAA
CTTTTCCATTTAACTCCTTCCTTTCTGGAGTACTCTCTTGTGTAGGGACTGCAGTTCTG
GCTGTTTGTCTCCGGATCCAAGTCAACAAAGAGAACAAGGAATTCAAGGATCTTGCC
CCCGAGCGCGCTTTTGCTGATTTTGTTCTCTGCAATTTAGTGCTTCATTTGGTGATCA
TGAACTTCCTTGGTTAAATTAGTTTGGTGTGGTTTTGTTGTTTCCAAGAGAACCTCGG
ATGATAAAGATCAATAGTATTACATATATGAGTTTAATCAAACAGTATTTGATTTAG
CAAATTACCGTAGTTCCATGTTGAGATGCTAGATATTTTTCTCTTCACTATGTTATTA
TTGATGAAGCCTCAGTACTACTCCAAAAACCAAGTTTATGTCTGGAATGGTGAATTT
GGCAAAATAATAATGACATGATTTTGTTTAA

Unigene sequence of the polymorphic marker UN3573

>UN3573

AGGTTATATTCCTGGTATAATTTATGCTCTTTATGCAATTATCTTTGTTGATCGTGAT
CAGTATTTTGATGAATATAGGCGTCCTTTGTATGCACAATCACAATACTAACACATA
GTGTGTGTGTGTGTGTGTGTGTTTGATTCCACGCGAATCGCTGTTGTTTATGTTGACT
GTTATGAATTGTCTTCAACTCTTGAATATGTAATGGATATCAGTTGAATTTGTTTCTT
ATATGTAATTCTTATTGAGATTGGACTTGG

Unigene sequenec of the polymorphic marker UN3291

>UN3291

CGGGGTGCTTGGGATAATGCCAAGAAATACATCGAGGCTGGTGCCTCAGAGCATGC
AAGAAGCCTTGGGCCAAAAGGGTCAGACCCACACAAGGCAGCAGTTATTGGTGACA
CCATTGGAGACCCATTGAAGGATACATCTGGACCTTCACTTAACATCCTTATCAAAC
TGATGGCAGTTGAGTCACTGGTGTTTGCTCCTTTCTTTGCAACCCATGGTGGTCTCCT
CTTTAAGTTTTGGTAATACAAGAGAAGAGCATCATCGTCCATCACTTCAACCAACCA
ACCAACCAACCATGGCGTCTCTCTTTTTTTGTTAGTTTTTTTATGGCAATTTGTTTTTC

TTTATTATCTTATTATTATCCCTTAATCCCCTCTCTTTAGATTTGAGATACTTTAATTA
GGCTGAATCTTTTCCGCGTGGTCATTTTTCTTGTAAAAGTCGAAGTTGATGACGATG
GCGATATCATATTTACTGTTTTGCCCCGCGTGGTCATTTTT

Unigene sequenec of the polymorphic marker UN0079.2

>UN0079

CCATATTTATAATTTTGATGAAAACTAGAGCAATTATCCTACTTTTGTCCACACGATT
ATCGTCGTCATCATGATGTCAATCGACCAATTCTACTTAAGATCCCGTGCTTGAGTA
CATTGTAAGTGATAACTGCTAGGATATTTGTAAGTGATAACTGCTAGGATATGATTG
GCACCAAAGGTTAGTTAAGTCAATTTGAATCCACATACTGCAATATAAAGTTGAGCA
ATTTACACAGGCAGTCAAACTGTACTGACAAACAGTATAAAATCCAGGACATAAAA
AAGAACCTACGCACACAAAATAAGCATATAAAACCAAATGGCCTAAATCATCTACA
TGCTAATAACACTGATGAGCCTAATATCTCTTCCAATTGTCCTTCCTGTTATTCCTAA
ATGCGCAGCAGCCGATGGAGTATACAATGACCAGGAAAATCAGGAATATGATGTTG
ACAACCGCAACCTTCTTCCAATCAGTCTTGAGATTTTGCAGTAAGCCAGCCTTGCAA
GATTGGCAATCGAAACACAGAATATTTGGATCGTTATTCCAAGTATTACAGTCAGGG
TTGGTGGAAGTAACATTTCCGGGCTTCGTCCAGCTTGTTGGGCTCACATAAGTAAAA
CCACAGTCCTCTGAAGGCTTACAACATCCAGACTGAAGAGCAGACAACTGCTCAGC
ATGAAACATGTCAACAGTATCATTCAAAAACTTCAAATGAAACTCAGAGCAAAGCT
TCCCTTTTTTTTTTTCTAATATGGAATACTTTTATTAATAAAAAGCAACCACATATAT
TACAGCACTAACCGTACACATGCACCATTTATTTAAATTTGGGAGGGTTTCCTTAGA
AATCCAACTCTTAAGTTGGAAACAAAGATTGTTTATTTAAAGAACAGGATAGGTTCA
CCCTGTGTTGTTTGCATGTTGTTGTTTTGGATATCAATATTAAGCACCGGCAATATTC
TTCAAGAAGTCTTTTGGGGTAATCTTAGAGAAAACGTCTTCTGCTTCAGATTTAGAG
GCAAAACACCATCCATATTCAATATCAGAATTTGGATAATGACCGCAATACTTCCCA
CTTCCCTTATTTGTACAATCGGCATTAGACTCACATAAATAAGGGTGTTCATCATTCG
TCCTCAATGAAGATGGATGTCTGCAATAACCTACAACTAGACCAACAGGGATACAG
CGACAGGCTGAAGAGCGACACGGTGGCATCTCAAATGGAGAACAAGCCCCATTACA
GTCTGCTGCTCCTGCGTTCTTTGTCTGGAATATACCGAATGTGGCAAACAAGACAAT
CAAAGAAGCGAGTTTAACATAAGCCATTGTACGATCAAAAGAAAACCCGCTACAGT
TGTTTCGAAATCACAACAACTCAACTTCATCGTTTCGGGTGAGACCATTGTTCGATG
GCGGCGGCGGCGGCAGCTTTAACAATGATATTGATGGCAGCCTGCATAGTGTGCAG
TACAGCGTCCTACAGGCCTCGCATGGTGGGTGGTAAAACAGAGATCGCTAACGTGA
GGACAAACGAGGAGGTACAGGAGCTTGGAAGATTCGCGGTGGATGAGTTTAACCGG
AGTGTGAAAGTACGGAAAGAAGGGGAAGGGGAGTTGAAGTTCGTGGAAGTGGTGG
AGGCGCAGCAACAAGTGGTGTCTGGAATCAAGTACTATATGAAGATATGGGTTACG
CGGGTGAAGAATGATGGTGCTGAGAGTGAGGATTCCACCATGTTCGATTCAGTGGT
GCTGGTTAAGCCATGGCTTTCTTCCAAACATCTTCTTCACTTCGCACCTTCTTCCCAA
TGATGATTAACAATAATGAATAATGTTATGTAATGTGATGTACATTAGCACCAGAAG
TTCTACCATGGCATCCATCATGTACTATTTTCAAGTATGTTAATACAATCCGGATCAA
TCAATCTATGAATTATAAG

Unigene sequenec of the polymorphic marker UN009

>UN0099

CTCAAAACAAACGCAAACGTCTCTTTTCTACTCCTCTTGAAACGATTCTAGATCGTTC
TCCAATCCACAACCACCTGTCACACAAACCCTTTCTTTTCCTTCTCTCAATCTTCTCC
TTCCGTGCTATTTTGCAGCTTCAAAAGGGAGCACAAGATGGCTGACTGGGGTCCGGT
GATAATAGCGGTGGTGCTGTTTGTGATTTTGTGTCCAGGTCTGTTGTTTCAAATACCA
GCAAGAGGAAGAATAATCGAGTTCGGGAACATGCAAACAAGTGGTGCCTCCATTCT
TGTTCACGCCATTATTTACTTTGGACTCATCACTATTTTACTCATCGCCGTTGGTGTTC
ACGTCTACACGGGGTAAAGTTAACTCATCGTACTGGATGAAAAGGGATCTACAATCT
TGATGCATTTTCTTTTTTGCTTTCTTAGTTTTTAAGATTGCCTTTATCTGTTTTTAAGG
TTTTCCATTCTGTTGTTTGTCCTTTTCACGTGTATGTATGCGTGTGGTCTAGAATATGA
ATTTGATCTGTGATACTGTTTTTTTTTTTTTTTTTTTGTGTGTACTTGAAAGCTGTTTT
GAAACTAAGGATAAGGATGATTTTGTTGTGTTGT

Unigene sequence of the polymorphic marker UN0106

>UN0106

TACGTTGAGAATCACATGAAGATGAAAATCCTCAAATCACAAACATGAAATTATTG
AATGAGAGATGAGTGCACAACCATATTTAACATCTAGATGACACAAGGATGGTCCA
AAGTCTGATTTGGTACCCAAGCAGCTGCCTTATTCCCAATGAAATGACATACCGGGA
CGGTTCCAGGAGCAACTTCCAGTGCTTCATAAAGCAACTCAGGATTCATACCTCTAG
TGTCATGGTGGCACACTGTTAAAGCGTTAGTTTTACTTCCGTCTGCGGCAACCAATG
AGACCACATAAGCGATTGTGTCACGCACTTGATGGCGAGAGAACTAGTCTCGAGTTT
TTTTTTTTTTTTTTTGAAGAAAGAAAAATCATGTATATTATCAGAAATTACAATCCAC
TTTGTTTATTGAGAAAACAAAACCTAAGGGAGAGAAAGAAAAGGGGAAGGGGGAG
ATTATTATAATAATAATAATAACTACCGTAAATCTGATCGGACTCAAACGGCGA
CGGCGGCGGCGTTGTCGTTAGTAGCGTTGGAAGGAGGAAGAGGAGGAGAGGTAGG
ATGTGAAGATCCATCGGTAAATCGGTGAGAATCGGGAGGAAGAACGAGAAGAGCG
TCGTCGGGATCATCGGAGGTAGAGAGATTGATGCAAGAACAGCGTTCGAGAGATGA
GAAGAAAGCAGTAGCGAGATTCATACCTATCCAGCTGGCCATGCCGTTCCAGCAAA
AGCCTTCACCGTCTCTCTCCATAATGGATTGATTGATTCCTTTGCTTCTTCTTTTGCTG
AAGAATGGAATTATTTGGGAGGAGAAAATTATGTGTTTTGGGGTGGCCGTGAGGGT
TACCAATCTCTTTTGAACACAGATATGGAACGAGAGCTTAATCACTTGGCTAGATTT
TTTGAAGCTGCTATTGCTCACAAAAAGAAGATCGGATTCAATGGGACTCTTTTAATT
GAACCAAAGCCACAAGAGCCTACGAAACACCAGTATGATTGGGATGCTGCAACTAC
AGCTAATTTCTTGCGAAAATATGGACTCGTAGGGGAATTCAAACTTAACATTGAGTG
CAACCATGCCACCCTATCTGGTCACAGTTGTCATCATGAGCTTGAAAC

Unigene sequence of the polymorphic marker UN0110

>UN0110

GTGAATATGGCTGGACTGCCAACATGGAAAGAATCATGAAGGCTCAGGCTTTGAGG
GACAGCAGCATGGCTGGTTACATGTCAAGCAAGAAAACAATGGAGATCAACCCAGA

GAACTCTATCATGGAGGAGCTGAGGAAGCGCGCTGATGCTGACCGCAATGACAAAT
CTGTGAAGGATCTTGTTCTCTTGCTGTTTGAGACTGCTCTTCTCACTTCTGGGTTCAG
CCTTGATGAGCCAAACACCTTCGGAAACAGGATCCACAGAATGTTGAAGCTTGGATT
GAGCATTGATGATGATGCAGCTGAAGCTGATGCTGACATGCCTCCATTGGAAGAAG
CTGATGCTGATGCTGAGGGAAGCAAGATGGAAGAAGTTGATTAAGTTGCGGTTTAA
TTATCTTATATTTTTTGAGCTTTTGTGATGACTCTTAGATTTTTTTTTTTACTTTTTTGG
TTATGTTTTTGCTATTTACTCTGCACTAATGGCGCTATTTATTAAATGCAAGTTGGGC
ATACTTTTATGGATT

Unigene sequence of the polymorphic marker UN0119

>UN0119

GAAGGTTGTATGTGCAAGTCTTTAAATTCAAACCAAGGCCTTCAAGGCTACAGGATT
TCAGGATAGCCATTAATAAATAGCTTTAACATGATTAATTTTAGCCATAAACCTTGA
TGACCTTGTGTTGGTCTATACTTTAGTTAATTAATGTACATTTACTTAATTTTAAGTA
CATGCAAAAATCACATGTTCCGAATAAAGACCTCAATTTGAGCCAAACTCTTCGTTC
CACTTCTCAAGCTCTTCCCATTTACTTTTGTTTAAACTTGGTCTAATGACACACATTG
CTTTCTTGAAATCTTCATACCGCAGTCCTCTTACCTGATTTGCCTTGACAGTGAGAAT
GTTTGAACCCAGCTCTCTAATTGGCATCATTGCAGCTTCTTCACACAAGGCTTGCAA
ATCACTTCCAGAGTATCCTTCAGTCTCTTTTACAAGCATTTCTAGATCTCTACTAGGT
AAGGAGAATGATTGACCCTTGAGTTTGTGTTTTAGCAGAAGTTTCCGAACATTTTCA
TTTGGTAAAGGTACGTATATTCTCTTAACCAGTCTTCTAAGAACTGCATCATCCAGTT
CCTGTGGCTTATTGGTCGCACCCCGAATTAGGAAATGGCGAAGTCGAAGAATCACA
CCGCTCACAATCAATCTTACAAAGCACACAAGAATGGCATCAAAAAGCCAAAGAGG
CATCGCCACACTTCAACCAAAGGGATGGATCCAAAGTTCTTGAGGAACCAGAGGTA
TGCAAGGAAGCACAACAAGAAAAATGGGGAAATTGCTTCCGATGCTGAGTAAAGTG
TCAATCTATAATGACCTTTTTGTATTTTCATCCAACTGTTTGTTTCTGAACTATATTTT
ATGTCAAATTTAGACTCTGATATTTCAGTACTCTGTTAGCATTTTTGCTCTATTTTAGT
CGCTCGTTATTTTATAATACATTTTGGTTGAAGTCTGCCTTTTTTATTGTGTTCGAAGT
TGTTGATAGTAGAAAGTTGATCTAAATTTACAGCTTGTAAACTGATGCTAGGAATTT
ACGTTTACAAGCCTATTTTAATGAAAAAAAAAAAAAAAAAAAACTCGAGGACGAA
TGATAGAGGTGGTATTGCAAGACTTGCAGGGACATCATCAGTTATAAACAGTATGCC
ATTGGATGTGGTTGCAGCTACATTCAACCTGCAGAGAAATGAGGCAAGGCAGCTCA
AGTCCAACAATCCTTTCAAATTTCTAATTCCACCGCGTCAGTCTCAGAACAGAGCTT
CGGCTTAGATTTGGCACCAAATCTATGATAATAATAATGAAAAGTATGAATAAGAA
TACTTAGGCT

Unigene sequence of the polymorphic marker UN0123

>UN0123

CACTAGAATTGTTTTCACAATTATCAGGAAAATGTTTGGTAATATTCTGTGATTCAA
GATGCATCAAAACGTTGGTTGTGAGACCTGCTTTGCCGGAGACACTACAACCATTGG
ATATAGAATGAGAACCAGGTTTATCAACAAGCATTACTTCAATATCAGCAGGAAGA
AAACCAGTATCAGCTTCACTGGATGCTTTATTCGTCTCATTAACACCATCCGCAATTT

TCTCACAAATACCAGAAGATACTGGTATAGATTGACAATCTCCAGGATTTTTGTTTG
TTTGGAGATCAATGGTTTTGGAAGAGCATAGTTCTTCATCAGCATTTGGCACACTAA
CAACACTTGAATTGTTTTCACAACTAACAGGCAAATCTTGGGTAATATTCTGTGATT
CAATGTTCATCAAGATGCTGGTTGGGAGACCTGCTAAGCCAGAGGCACCATCACCAT
CTGGTACAGAACCATTGTCAAATTCAGTATATACTTCTTGTGATCTAATACCGTCTGA
TTGAGCACAGTCGGTCTTAAATCTCAGTTCACCACTATCAGAAATCTTGTGAGAATC
ATGGTAACAGTCAGCAGAATCTTTTGATTCCCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCGGGCAATGCACACCATGCAGGGAGGGAACTGGATGGCT
TTGGATGATCATGGAAAGAATGAAAGTTGGGAATGCCAAGCTAGAAGAAATTGATA
TGCTTCAGGAGGTGACTAAGCAAATTGAAGGGCACACAATCTGCGCCTTGGGTGAT
GCTGCTGCATGGCCAGTGCAGGGACTTATCAGGCATTTCAGGCCCGAGCTTGAGAG
AAGGATTAAAGAGAATGCACAGAGGGAGTTGCTGCAGGCCACTGGTTAGGGGTGTG
ACTACTGGTGGATTGCAAAAATCAAAATAAGGCAGTGCCAATCATTTTGGAAAGAT
AGGTGGTTCAGGTTACTCTATTTACTCTTATGCTGTACCAATTATGGCTGGACCATGC
CAGAGAAACCATCCAAGCCTGTATAAACTGTGCTCCACCTAAACTTGTCATTTGGCA
CATTTTCATGTAATTTGTTAATAATATTTGTATACTGTCTTTATTTTTGAGAAACGTC
ATGTTTATGTTTGTTATT

Unigene sequence of the polymorphic marker UN0146

>UN0146

CTTGTTGTCACTCCCTGCTGCTACTTCAATTTGCGTCTCTCAACTCAAGAAAAACAAG
CAAATAATGGCAGTCACATTATATAACCTTAAATCTGAATCTGGGTTGAAGAAACTT
GACGAGTACCTTCTCACACGCAGTTATATCTCTGGGTATCAAGCTTCAAAGGATGAT
ATCACTGTCTATTCAGCTTTGTCATCAGTTCCATCACATGAATTTGTGAATGTTGCTA
GGTGGTACAAGCACATTGATGCTTTGTTGAGAATTTCTGGTGTTTCTGGTGAGGGAT
CTGGTGTCATTGTGGAATCTTCTCTTGTGGCTGAAGAGGCTATTGCCACTCCTCCAGC
TGCTGACACCAAGGCCACTGAAGCAGAGGATGATGATGATGATGATGATGTGGATT
TGTTTGGTGAAGAGACTGAGGAAGAGAAGAAGGCAGCTGAGGAACGTGCAGCAGC
CGTGAAGGCATCTAGCAAAAAGAAAGAGAGTGGAAAATCATCTGTATTGTTGGATG
TGAAGCCATGGGATGATGAAACCGACATGAAAAAGCTTGAAGAAGCAGTGAGGTCT
GTTCAGTTGGACGGACTGTTATGGGGCGCATCCAAACTTGTTCCTGTTGGTTATGGT
ATCAAGAAACTTCAAATTATGATGACTATTGTGGATGACCTGGTTTCTGTCGACAAT
ATGGTTGAGGATTATCTTACTGTCGAGCCAATCAACGAGTATGTCCAGAGTTGTGAT
ATTGTTGCCTTTAATAAAATATAATCTGCTATCCGCGTGACATGGAGATAATGATAG
TGGGGAGAGTTTTGCAATAATTAGAGATGATAATCAAGGGTTACTATTAAGCTGAG
ATTTTTGTTTTCTGGAAACCTATCTGTTACTGTTGTTTGATGAATTTTGTCTTGTAATT
TTATCACTGCTATATGGATTGTGTTATAAATTATTTAAGTTTTAAGAATTGAAGTTGT
GTTGTGTATTT

Unigene sequence of the polymorphic marker UN0225

>UN0225

CTTCACGGGAGAGAGACGACGGCCACACCCACCGCCAGCAAACATCATCCACCATG
GCTACCGCCTCATCCCGTGAACTGTCTCAGAAGGAAGCCGACATCCAAATGATGTTG
GCTGCCGATGTTCACCTCGGCACCAAAAATTGTGACTTCCAAATGGATCGTTACATA
TTTAAACGCCGAAATGATGGTATTTACATTATAAATCTTGGAAAGACATGGGAAAA
GCTGCAACTTGCAGCTAGGGTTATTGTTGCGATTGAGAATCCGCAGGACATTATTGT
GCAGTCTGCTAGGCCTTATGGGCAGAGAGCTGTTCTCAAGTTTGCTCAGTATACTGG
AGCTCATGCTATTGCTGGAAGGCACACTCCTGGAACCTTCACCAATCAGCTGCAAAC
TTCCTTCAGCGAGCCTCGTCTTCTCATCCTCACTGATCCAAGAACCGATCACCAGCC
AATCAAAGAAGCTGCTCTTGGAAATATTCCTACAATTGCATTCTGTGACACTGATTC
TCCTATGCGGTATGTTGATATTGGGATTCCTGCCAACAACAAGGGAAAGCATAGCAT
AGGTTGTCTCTTTTGGCTTCTGGCTAGGATGGTTTTGCAGATGCGTGGTACTATTCGC
CCAGGCCTTAAGTGGGATGTGATGGTGGATCTATTCTTCTATAGAGAACCTGAAGAG
GCCAAGGAGCAAGAGGAGGATGAAGTTCCTCCCCCAGAGTATGTCATTGCCGACTT
TAATGCGGCTGTTCCATCTGACGGTCAGTGGCCTGCTGCAATTGATCAACCTTGGGC
AGATGCTGCTCCTCCTCAGCCTATTCCAGCAGTTCCAGCAGTCAACTGGACAGCCCC
AGAAGCTGTTGCAGTTGCAGGGGACTGGGGTGACGCAGTTCCAGCACCACAACAAA
TTCCCACTCCCGGAATTGAATCTGTGCCAGCAACCGGCTGGGATTAAACTAGATTAT
GATCTTATGATGTTCCATGCCCCTAAGTTTACATTTTCATTCCCAAAGTAATTTTGAG
TCAGACTTTTTTTTGATAGCATAGGGACATTTTTCTTTAAGTTTTGGTATGAATGACA
GAAATATTGTTTACGACCTGTGAGATATATTAAGTAAAATTGTACTTTTATTAGTGA
ACATGTTGCAATGCTTTTAGCCTCCAAAAAAAAAAAAAAAAAAAAAAAAAAAACT
CGAGACTAGTTCTCTCATTTCTATCTCTGACTCTGAAACAAAAATCCCAAATTCTCTT
CAAGAAAAAGAAAAACCCTAGTGCGATAATCAACCATGGGGAACACCGAAAAGCTT
CTGAATCAAATCATGGAATTGAAATTCACCTCGAAATCGCTTCAACGCCAAGCAAG
AAAGTGCGAAAAGGAAGAGAAATCA

Unigne sequence of the polymorphic marker UN0230

>UN0230

ATTTATTCATCAAAGTTGTTTTTCCTTGAGTTGAAGATTCCATAAACATGGCTTCCAT
TGCTGCTTCAACTGCAGCTGCTTCACTTGGAATGTCAGAAATGCTTGGAACTCAAAT
TAAATTCAGTGGTGCAACAAGGTCTGTTCCTTCTTCTTCATCTGCTTCTAGTTTTAAA
ACTGTTGCTCTTTTTTCGAAGAAGAAGGCGGCTCCGGTGAAGCAGAAGGTGGTTACT
CCAGCCAACGAGGAGCTCGCCAAGTGGTATGGTCCTGACAGAAGGATCTTCTTGCCT
GACGGTCTCTTGGACAGGTCTGAGATTCCCGAGTACTTGACTGGAGAAGTCCCCGGA
GATTATGGTTATGATCCTTTTGGTCTAAGCAAGAAGCCTGAAGACTTTGCTAAATAT
CAGGGATATGAGTTGATTCATGCAAGATGGGCAATGCTTGGTGCTGCCGGATTCATC
ATTCCTGAGGCTTTCAACAAATATGGAGCCAACTGTGGTCCTGAGGCTGTTTGGTTC
AAGACAGGAGCACTTCTTCTCGATGGAGGCACGTTGAACTACTTCGGAAAACCAAT
CCCCATCAACCTTATTCTCGCTGTTGTTGCTGAGGTTGTTCTCTTGGGAGGTGCCGAG
TACTACAGAATCACCAATGGACTGGACTTGGAAGACAAGCTTCATCCAGGCGGTCC
ATTCGATCCATTAGGTCTAGCAAATGATCCAGACCAAGCTGCAATCCTAAAAGTGAA
GGAGATTAAGAATGGTAGACTTGCTATGTTTGCCATGCTTGGTTTCTTTATTCAAGCT

TATGTCACAGGAGAAGGACCTGTCGAGAATTTCGCGAAACATCTCAGTGACCCTTTT
GGCAACAATTTGCTCACTGTCATTGCTGGAAATGTTGAGAGGGCTCCAACTCTGTGA
TGAATCAAATCATTTTCATGCCTTTTTTTTTTCTTAATCCAATCTTGAATGTGTACAAT
ACATCTCACTGAAGGCATTTTGCTTTATTTAATGGAAGCAAACTCACTCTTCATTTTT
AGTGCATGATTATTCGGCCCGTAATTTAAATTTGACGATCTTGAAAATTTTCTGATTT
TCTTCATCCGGTTAAATAAATATTTGCC

Unigene sequence of the polymorphic marker UN0281

>UN0281

CTAGAATACAAAGAAAAAAATTTAAGATGCACATTCGCAAACTTTTTCTCTTGGGAC
TTTTATTCATTACGTTACTCCTATCTTCTGGAGCAAGAAACGGTATAGAAGAAACAT
GCATTGGAGGGATATGTTATAATTGCGGCGCAAGATGTCTTTTTTTAGGATTTCCGA
GAGGTGAATGTAATTCTCGTTCCCTATGTTGTTGTAGTCCCACAGAACATTTTTAAAA
TAAATTACATTATTATAAATACTTAATAATATTGGATAAAATAAGTAACACACATCT
TCCTCCAAGGTTTTTATTTTTATATTTGAACCATAAATATTTAATGTCTGGCTTGAGC
AGAAGAATGACTTCTTCAACTTAAAGACAGGTAATTGGAGTGAAAATTGTTTCCATT
TAAATATTATATATTATGGGTGTAATTATAGTTTATATTCAATGAAATATAAGTATTT
TATGTTAAAAAAAAAAAAAAAAAAAAAAGGAACCCTCTGAGGCAAGCTATGGCAA
CAGGAAATGGTGGAAGGGTTACATGCTTCCAGAGGGATTGGCTACGGAGGGATTTC
AATGTAATAGGGTTTGGGTTGATAGGATGGTTGGCACCTTCTAGTATACCGGCTATT
GACGGTAAAAGTCTGACTGGGCTTTTCTTTGATAGCATTGGAACTGAGCTTGCTCAC
TTCCCTACTCCTCCTGCTCTCACTTCACAGTTTTGGTTATG

Unigene sequence of the polymorphic marker UN0536

>UN0536

CCGAATGAGAGCAACTCTTTCTTCTTCCTTATTCTCCCTCTTCTTCGCAATCTCCACC
GTTCTTCAATCTTCCGGCGACTGTTCCGATTTCGATCGCCCCCAAATGGCTACCAGA
CTAGGAGGCATCCACGATTCCCCCAGCTCTCAGAATTCCCTCGAAACTGAATCCCTC
GCTCGATTCGCTGTCGATCAACACAACGCCAAACAGAATTCACTTCTGGAGTTTGCA
AGAGTGGTCAAAGCACAGGAACAGGTTGTTGCTGGTACAATGCATCACCTTACTATC
GAGGCTATTGATGCGGGTGAGAAGAAGATCTATAACGCCAAAGTCTGGGTAAAACC
CTGGCTCAACTTTAAAGAACTTCACGAGTTCAAGCATGCTGCTGATGGTGATGGACC
TTCATTTACTTCTGCAGATCTTGGTGTGAAAAAGGATGGCCCCAAGCCGGGGTGGCA
GTCTGTACCAACAGAAGACCCTGCAGTTCAGGATGCAGCAAATCATGCTATTAAGA
CCATCCAGCAGAGGTCCAATTCACTAGTGCCCTATGAACTCCATGAGGTTTCTGATG
CAAAGGCTGAGGTCATCGATGATGTTGCTAAGTTTGATTTGCTTCTCAAGCTCAAGC
GAGGAGAAAAACAAGAGAAGTTCAAGGTACAGGTGCACAAGAATAACGAAGGGAG
TTTCCATCTTAATCACATGGAAGCAGATCATTCCTAGTAATCTTCATATAAGCTTGGT
CATGGTAGCATTATAGGCCTGCTTGGACCCTAAATTTGCACTTTTTATATATATATAT
GAGATGTGTATCATGTATGACTCACACATAACTAGTACGTTTGGAAATTGCCTTTGT
TTTATATGTCTCAACAATTATATCAGTGGTTGCCCTTTTCTT

Unigene sequence of the polymorphic marker UN0538

>UN0538

GCAGAACAGTCTCTATGGTTGATACTCAGAAGACAATTCAGGAGGATGATGAAAGT
ACTAAGCCAGTAGCCATCAAATCTGATGAAATTGTTGAGAATGAGAAAGCTGGCTT
AGAAGAGGCATCAGAGAAACTTGTTACACAACTATCACTAGGCATGGAACAACCGA
AGGAGACTCCGTGTATCGGTGAATGCGCATCATCTTTACCATGTTGGAGTTTATACC
AAGGCCTTCCAGCTTTGAGCCTCAAGCCATGCAATCATCAGATTTTAAGTCCTGTGC
CTCTGAGGCCTTGTTTGAAGGTAAGAACTCGAGAGGAGGAAAGTTCTTGCACCGGTT
CTAATACCGGATCAGTTTGTGATATGGAAAAACAAAGCAAGAACAATTCATCAGAT
ACCGACGATACTTGGAGTCAAAATCATCCTCATGAAGGAGTTTTAGTTAAAAAATCT
GGAAGAGGGTTTGTACCATATAAAAGATGTTTATCTGAAAGAGACGATAATTCTTTA
ATTGTTGGGTTGGAAGAAAGAGAAGGGCAAAGAGCTCGTGTGTGTTCATAGCTTTTT
ATTCCATTTTTTTTTTTTGCAATGTTCATTGTGGTTGTCAAAGAAAAAAGTTGCTGAG
AATAAGGAACTGATTTTTGGTAGCTGTGATCTAACTGCTAAGTATCTCAGATTTTCA
ATTCTTTTTGCAATGTTATGTAATATGAATATTGTCCATTTGATGTC

Unigene sequence of the polymorphic marker UN0575

>UN0575

CTTCTCTCTCTGCGCGCCTCTTCCGTTTTCAACAGAGACGCCGCAGAGAAAAACTTT
ACACAAATCTAAAATCTATCTATCATACATTACTATCTTCGTATCATTTTGCGATATG
GCTTCCTCTAAGCATTCATTCGGATTCGGAATTGTGGCTATTCTCGCCACTCTCATTT
TTGCTCTTTCCTTCCCCGCAGCTGTTCACGCTCAATCTCCTTCCCCTGCACCTGCTCCT
ACCAGCGACGGTTCATCGGTTGATCAAGGAATAGCTTATTTGTTGATGTTGTTGGCG
TTGGTACTCACCTACATCATTCATTCGGCTGATATTTCATCTACCTTGTGAATTGCAT
GTTGTTGATGTGTGAGAGAGATGTGTTTTTTTTTTTTAATTTATGATTAATAATTAAG
CGCGTTTGTAGAACGCGGAGGAGGAATGGTATATGTAATGTAAGAATAATGATATA
GATTGAGAGGTATGTTTGTTTTTCTAGTGAATTAAAGAATGTGATGCGTGAGTTTAG
AGGCTGTATTGTAGTTTAGCACCAATGTCTCATTTGTGTAAGTGATGAAGGATCTAT
GTATTATTATTATTGCAATCTGGTCAATGTGATTTT

Unigene sequence of the polymorphic marker UN0748

>UN0748

GTCAATGATGTACTCAATGCTATTGATCATGTTATAGACTTGGGACTTGCCAGTCCA
TCAAAGATTGCGGTACTTGGTGGTTCACATGGTGGCTTTCTGACAACCCACTTGATT
GGCCAGGCTCCAGAGAAGTTTGTTGCAGCAGCTGCTAGAAACCCCGTTTGTAACCTT
GCGCTGATGGTTGGTACAACTGATATTCCTGATTGGTGCTTTGTGGAGACCTATGGA
ACCAATGGGAGGGATAGGATTACTGAAGCACCTTCAGCAGAGGATCTCACTCTATTT
TATAGCAAGTCTCCAATTGCACACATCTCAAAGGTAAAAACACCAACAATTTTTCTA
TTAGGTGCCCAAGATCTTCGTGTTCCAATTTCAACTGGACTGCAATTTGCTCGGGCTT
TAAAGGAGAAAGAAGTACCGGTTAAAGTCATCGTGTTTCCAAATGATGTTCATGGA
ATTGAAAGACCACAATCAGACTTTGAAAGCTTCCTTAGCATTGCTGCGTGGTTCAAC

AAGTACTCCAAGTGAGTAAAATTTTCAGACACCGGCATCAAATCATATGATTTCCTG
GATATTCTTGTGTAGCTGTATTATGTTGCAGACTGTATTTTAAATGAGTATGATAGTG
GAGCGAACTGCATTTGTCATATGGGCTTTATTTAAAAAAAAAATTTATAAAGTAGAA
CATGACACTGAATATTTGAATATGAATAA

Unigene sequence of the polymorphic marker UN0755

>UN0755

CAAAATCAAAAGCTTTATTTTTCATCTTCAAGATATTAATGTTGAATATCATAACACC
AGTAATGATTCATGCATGTGGTACATGCACACCAAATCCACCACCTTACCACCACCA
CCACCGGCCAAGCCATCCAAAACATCCACCGCATCACGGCGGAGGAAAAGGACGTC
CAATAGTGACTCCTCCTCCAGTTGTTGTCGTGCCACCGATAATCGTCACTCCACCACT
GCTACCACCTCCGACTGTCATATACCCTCCACCAACAGTCTCTCCTGTTATTCCGCCA
CCAGTAGTTCAACCAACTTGTCCAATTGATGCACTCAAACTTGGAGTTTGTTTGGAT
GTTCTTGGAGGTCTTGTTCATGTTGGAATAGGAAACCCTGTGGAGAATGTGTGTTGT
CCTGTTATTCATGGATTGGTTGATCTCGAAGCTGCTATTTGTCTTTGTACTGTTATTA
GGGCTAAGGTTCTTAATCTGAATATTTTCCTTCCTCTTGCTCTTCAAGTTCTAATCAC
TTGTGGGAAAACTCCTCCTCCTGGTTTTGTTTGTCCACCTCTCTAAACTATAAGTAAA
GCTCTACATGCATGCATGCTGCATGCATTATCCATATATACTTAGTATTATCAAGCTA
ATTAGTAGTTTAGTTAATGTCTAGTTATTTGCTTTCTAAATTATGCGTTTTTACCTTCT
AATTAGGATTTTCGGGTTGATATGTAATGTGTGTAAGTACTATATTATGCATTAGTGC
ATTATCTCCTTTTATCTATTTGTCTTTTTAT

Unigene sequence of the polymorphic marker UN0861

>UN0861

TGGACCTCAAATTTATAAAACTACAGCTACACTATTCATAAAACTTACACTTCATTC
ATTAATAGTACTTTCCTCAGCTAAATAAATGAGATAAATAAATGAGATATCGCGAGT
TCGAAAGCGTCATCTACGCAACTAGTTAACTAAATAATTTAATTGAGATTATAATAA
TGGTTTAGTAGGACATTTAGTTAGGTATTTTGTTATTTAAGCACTATCAAGCACTTCA
GTGAACTTCACTTCACTCAACAATGTCTCCAAACAAAGCCTTCATCTTCTTAGCTCTC
TTATCCTTTTCACCACAGCTCTTCCTAACCATTTCTTCTGCAGAACAAGATAATGGCC
TTCTCCTAAACTACTACAAAGAATCATGTCCACAAGCTGAAGAAGTCATCAAAGAA
CAAGTCAAACTTCTCTACAAACGCCACAAGAACACCGCTTTCTCATGGCTCAGAAAC
ATTTTCCACGACTGTGCTGTTCAGAGTTGTGATGCTTCTTTGTTGCTGACATCCACAA
GAAGAAGCTTGTCTGAACAAGAACACGACAGAAGCTTTGGTTTGAGGAATTTTAGG
TACATTGATACCATCAAAGAAGCTGTTGAAAGAGAATGCCCTGGTGTTGTTTCTTGC
TCTGATATCCTTGTTCTTTCAGCTAGAGATGGAATTGTTTCGTTAGGAGGTCCTTATA
TTCCATTGAAAACTGGAAGAAGAGATGGCAGAAAGAGTAGAGTGGATCTGTTGGAG
GAGTACCTTCCTGACCACAATGAATCTATTTCTTCTGTTCTTGACAAGTTTGGTGCCA
TGGGAATTGACACTCCTGGAGTTGTTTCTTTGCTTGGAGCACACAGTGTTGGTAGAA
CTCATTGTACAAAACTAGTGCACCGTTTATACCCAGAAGTTGATCCATCTTTGAATC
CAGATCACATTCCACACATGCTAAAGAAGTGTCCCGATTCAATCCCTGACCCTAAGG
CAGTACAGTACGTGAGAAATGACCGTGGTACCCCCATGATTCTGGATAACAATTACT

ATAGAAATATTCTCGACAACAAGGGTCTTTTAACAGTGGACCATCAACTAGCACATG
ACAAAAGAACAAGACCCTATGTGAAGAAAATGGCAAAGAGTCAAGAGTATTTCTTC
AAGGAGTTTTCTAAAGCTATTACATTGCTTTCTGAGAATAATCCTCTAACCGGTACT
AAAGGTGAGATTAGAAAACAGTGCAGTGTTGCTAACAAACAACACCATGATGAGCC
TTGAATGAAAATTTCACGGTGGAAGAAGAAGAAGAAGAAGAAGAAGAACAAG
TGATGTGTAAGATATTGTGGAGAGATTAATAAAAGTGTTCCTGGAGATTGAAGAACT
TAGATGCTTTTAATTTTTAATTTTAACTTTGTTTTTCACTTATTTAATATTAGTATTAT
GGTAGCTCAATGTTGAGCATGTTGATGATGTGATGTATGGCATTTTATGTGGTCCAT
GGATGACACATAGTTACAATGTTTATGAAATTTGTGCACATCAAGTTTGTGTGTTTTG
AGCTGTACGATCCTTCAACGGTAATGTTCTTCTTTAGGAACAAGCACATCATGATAG
ACCTTGGAACTGGAAACAACAACAAGATTAATTGGGCTATGAAGG

Unigene sequence of the polymorphic marker UN0931

>UN0931

CCAGAGCCAGTTATACAAAATCACAGGTCTATATAATATAGCACTTTTTCTCCTTTTC
TTTTATTCCATCCTCTCACAAATCAAAATGAAACCTGTGTTTGCAATATTCGTTATGT
GCCTTGTCCTCAGTTCCTCGTTATTGGAAGCTGCATTAGCTGGTGGTTCTGGTATTTG
TGACACCAAGTGTGGAGAAAGGTGCTCGAAAGCTTCGGTGCAGGATAGATGCTTGA
AGTACTGCGGAATCTGTTGTGAGAAATGTAATTGTGTGCCATCTGGAACTTATGGTA
ACAAAGATGAGTGTCCTTGTTACAGAGACATGAAGAACTCAAAGGGACAAGGAAAA
TGCCCTTAATTTGCTCACACATGTTTCCTATACTTTCTACTCCACAAAACTTGCATAT
GTATCTCTAAGCTTCATTATGTCATGTTCTAAATATTGTACGTTGTTGTGTAAGGGAA
ATTTCATCATGTTATGTGAATGTTAGCATGTGAGGATTATAATAAGTTCACAACCTC
ACTCAACGAATAAGAATATTTTATTTATCGGCCAAAAAAAAAAAAAAAAAGTGGTTT
TGGGTGATGTACAGGGCTAAGCAAGATGCTCCTGTAGTACTGGGCTGGAGGCATCC
CTGGGAGGGCCATGATGATCATGGAAGCGGCCATTAATTTTCTTAGGCTCCAGCTAA
ACATGAACATCAGAACTTGAAGAATCTATGTTCCTGAATGCGCGTTAATCAAATGGT
TACTTTGTTTCATTCAATAAATTGGCTATAAAAGATTCTTTTTACTGTGTTTGAACAC
CTTACTCTTTAACTTGGATGAAAGTTTATGGTAGTTGTTGTTTAGACTATTTATCCAT
GTTTT

Unigene sequence of the polymorphic marker UN0953

>UN0953

CTTGTTTAGCACATCACTCTGAATTAGACAGGTTCAACCAATGCCAGCTTGACAATA
TCAATGCATTAGAACCAGATCACCGTGTTGAGTCAGAAGCTGGTCTCCCTGAAACAT
GGAATCCAAATCACCCTGAGCTACAATGTGCCCGTGTTTTTCTTATCTGACGCACCA
TTGTCCTAATGGCCTTCACTTGCCTTCTTATTCACCATCTCCACGGTTGATTTTCATCA
TCCAAGGTTATTATATATCAAATTTACATAATGATTAAATATTCATTATTTGCTAAAT
ATTTTATGCATTCAATGAATTGTTTTGCAACTGATTAGTGTTTTTTTTCTATATAGGG
AAGGCAGTACTTGGTCTTGCAGTTACTGGTTGTCCCGAAACTTACGAAGAACCACGC
TCATAATCTAGACAGCGTGACAGCCACCAGAAGATTCGACGCTTCTCTAAAGGTGAT
GTCATTGTCATTCCACCTGGAATTCCTTACTGGACCTATAACCATGGTAATGAACCTC

TTGTTTCCATTAGTCTTCTTGACACTTCCAACACTCTAAATCAGCTCGATTCAACTCT
GAGAGTATTTTACCTTGGCGGAAACCCAGAGGCAGAATTCCCCGAAACACAGGAAA
AACAACAGGAACAAAACGAAGGTAACAGCGTGTTGAGTGGCTTCAGCGCAGAGTTT
TTAGCACATTCACTCAACACCAAGGAAGAAACAGCCGAGAGACTTCGATCTCCACA
TGACAAAAGGAGTCAAATCGTAAAAGTAAAGGATGGTCTTCACATTATCAGTCCTG
AGTTGCAAGAAAAAGAAGAACAAAGTCACAGTCAAAGAGAGGAGGAAGAGGAAGA
AGAACTAGAACAAAGATACCACAAACATAGTGGAAAAGAAGAGGAGGGTGAGGAT
GAGGAAGAAGAACAAGAACATCGAGACCACAAACATAGCGAAAAAGAAAAGGAG
GATGAGGAGATGAACCTCGCAGCCATGAGATTCGCAGAAAGTAAAAAAAAAAACA
CAGAAGAGAAGAAACGAGAATCACATGGACGAGGAGAAGAAAAACAAGAACAAG
AAAATACAGAGAAAGAGGAAGAAATACAACGTCAACACAGCAAAGGAAGTAAGAA
TGGTTTGGAAGAAACTATTTGCACTGCAAAGATTCGCGAGAGCATCGCTCGCCCTTC
ACGTGCCGATCTTTACAACTCACGTGCCGGCCGCATCAGC

Unigene sequence of the polymorphic marker UN0982

>UN0982

GACATCTTGGGCTAGCTTACCGGTGGCAGTTTGTTGAGTGATCGGGTTTGCGGAAAT
AAGCACACACGTGTTGTTATGGCAAGTGAGAGCATTGGCTCTAGAGCATTGTGTGAA
AGAATTTAAAAGAAAGAAAAGAAATGGCAAGAAGCATGAACTTAGCTTGTGTTGCA
TTGGTTATGTGCATGGTAGTTATTGCGCCTATGGCCGAAGCTGCAGTCTCATGTGGA
ACTGTAACCGGTGATCTTGCTCCATGCATTCCTTATCTTACAGGTGGTGCTGGTCCTA
CAGATTCATGCTGTGCAGGAGTGAAGAAGCTTCTTGCTGCCGCCCCCACCACGGCTG
ATCGTCAGGCTGCCTGTAACTGCTTGAAAACAGCTGCCGGTAATATTAATAATTTGA
ATCCAGGCAATGCTGCTGCTCTCCCTGGCAAATGTAATGTCAACATTCCATACAAGA
TCAGTACCACCACCAACTGTAATACCATTAAGTTTTGAAGATGATGGTGCGGTTTCA
AGGTTATCATATGGAAACTTCTCACTAGTATATGAGAGTTTACTACTAAGAATAATG
TCTAAATGAGAGTGATGTAAGATCATCTTACGTGTGGTCCTATCCTCTTGTACTAGAT
CTATATGCTGTATTCAATGTCTCTTACTATGTGTTGTTGTTGGTATCAATCTACATAA
TGACATATATTAATTTGAAAAAAAAAAAAAAAAAACTCGAGACTACAGATTTGGAG
TATTAAACCTGGATGGGGGAGTAGGCCGGATATACATATTGAAAAATGTCACGAGG
ATGATATTACTAGTTTGAAGTTTTCTACAGATGGG

Unigene sequence of the polymorphic marker UN1014

>UN1014

TCCACCTCATGCAATATACCAAGTAGATCCCAGCATGGCCGTCCCCGCCACTCAACC
ATCTCCTCCGCCGCACTTAGACTTCCATCCGTCAAAGGAGAGTATAGACGCAGCTAT
TAGTGATGTATTATCAAAACCATATTTACCATTGCCTCTTGGCCTTAAAGCTCCTGCA
CTTGAAGGTGTGATGGGTGAGTTGCAGAGACAAGGAATTCCGAAAATTCCACCCTCT
TGTGCTTGAATGAAAGAGAATGGTTTTGGTCCATGAAACCCTAATTCGACGAATTTT
CATATTCTCATTTCTTTTTGTTTGTTTGAACTCTTTTTGTTTGTTTGTTTGAACAAAAA
GAGTGAGGGACAATAATATTAAGAACTTTCTGTGTTCATATGATATATCTAAGCAGC
GCAAAAAGTTTAACTCGAGCTTTTGGTATTATATTGTTTCGGATGTAAAGACAAAAT

GTTAAGATGATGTGTTTTTCAACATTAAAAAAAAAAAAAAAAAAACTCGAGACTAG
TTCTCTCTCTCCTTTTTTCTCTTAGAATTCTTCATGGCTCGTTCAATTTCTTTGGTTTCC
ATTTTTGTCTTCTTTCTTCTTCTTGCGGCCACTGGGCCAAGTATGGTGGCAGAGGCAA
GGGACTGTGAATCTCAAAGTCACAAATTCAAAGGAACATGTTTGAGTGATACCAAC
TGTGCTTCTGTGTGCCAAACAGAACGTTTCACCGGCGGACACTGCCGTGGATTCCGT
CATAGATGCTTTTGCACTACACATTGTTGATGAAAGAAAGATGATGGATCCATCACC
TTTTTCTATTTCTGTGTGTTTTGAATAAAGCTACCTGGCTACCCATTTTGTTATGGGTT
TCTTTCTTTGTATCTTGTTATAGATCTTTTGATCTTACGTGTGGAAATTAATAAAAAA
AAAAAAAAAAAAAACTCGAGACTCAATTAACCAAATCAAAAGCTTCTTGCATCACTT
TCTTCACTTCCATCCAAATGGCTCGTACCAAACAAACCGCTCGCAAATCCACCGGAG
GCAAAGCCCCTAGGAAACAACTCGCTACCAAAGCCGCCCGCAAATCTGCTCCCGCC
ACCGGAGGAGTCAAGAAGCCTCACAGATTTCGTCCCGGAACCGTTGCTTTGAGAGA
GATCAGGAAGTATCAGAAGAGCACCGAGCTTCTCATCAGGAAACTTCCATTCCAAA
GACTGGTTCGTGAAATCGCCCAGGATTTCAAAACAGATCTCCGATTCCAAAGCAGTG
CCGTTTCTGCTCTTCAAGAAGCCGCTGAAGCTTATCTTGTTGGTTTGTTTGA

Unigene sequence of the polymorphic marker UN11828

>UN1128

CACCAACAACAACAGCAGCAGCAATAACAGAAGAACAAAAAGGAAAGGAAAAAA
AAAACATTCAACAAACCAAGTCAAATCATAATCAACCTTAAATGATTTTACTGCGAC
CAAATCAAACTCAATAAGGAATCAAAGAAGAAGAAGTAGAAGAAGAAGAGGAATT
AGGGTTTGAATTGAAGTGAATTCAAATGCCGGAAGAGGAGTTGGTTGATATCAAGT
TTAGGTTGTATGATGGGTCTGATATTGGACCGTTTAGGTATTCTTCTAATGCTACTGT
TGATATGCTCAAGCAAAGGATTGTCTCTGATTGGCCTAAAGGCAAAACAGTCGTGCC
AAAGTCAGCAAATGAAGTGAAATTGATTAGTTCTGGTAAAATCTTGGAAAACAACA
AGACTGTTGGTCAGTGTAAAGCACCATTTGGGGATATTGCGGGGGGAGTTATAATCA
TGCATGTTGTTGTACAGCCATCTCTAGCAAAATCTAAAGCTGAAAAGAAGATTGATG
ATTCATCGAAGAAGGTTGTCTGTTCGTGTTCTATATTGTGAAGACAATTTGTAAGTTG
GAGACGCTATCATCGATCACTTGTGAACCACAGAAACCATCCCAACTGAACAAAAA
TCCTTCTATTTTCAGCATTCCTGTGTAAAGTTCGTATACAAGCAATTATATGTTTATA
GAAAGTTTGTGTTAATGGTGACTTGTGAAGAAGATGTCATTCTTTTATTACAACGTCT
A

Unigene sequence of the polymorphic marker UN1583

>UN1583

ATTTCGCATTCATGTCGGCGGTACGTCCCCAAATTTTCCGATTTTCTTCCCGATCGTC
GTATCGTATCATCTGTTGTTGTATATGATTCCCAATTGTTCGTTATAGATTTTTTGAAT
TTCGTTTCATAATGATTTTGTTCTCATTTTACCTTTTCTTAGCTTGAAATTTTAGTTTT
ACGTTTCACAATTTAAAACTATTATTATTATTATTGATTTCATATTCTTATGATTACTT
TTTATAGATGTTCATGAATGAAAATATGGATAGTTTCCTTTGTATTTAAAGGTTCATG
ATGCAGAAAATTGATAGCAAATATGTGTTTTTCTGGAAAATGTTTGGATTCTAAAAT
GGAAAGTGAAAGAATAAAGTGGCTTTATTGTTGTGTTCTGGTTTGTCTTTTGCTTGTG

GAGACGTAGTAAATAAGGGTCTCTTTTGATTAAGAACTAATATAGAGTATATAGCAC
AAATACTAATCAAAATAAGCACTTGTGAATAAACTTGCATAAGTTGTTTTTCTAACA
AGAGTTAAAGTAAAATTGAGTTATTTTTATTCGTGCTATAAATTGTTTTCACAAGTTA
TCTTTGAAAATTCATAAAAATAAGCTTAAAAAAAGTTTATGAATCTTGTTATAAGCT
ATTTTAGTAAGCTTTTCCAAACAGTGTCATAAAACTTATGGCATAAGATAAACTCAA
ATAAGTCAATCCAAACATATCATAATTCAGGGTGTTGCTAGTGTT

Unigene sequence of the polymorphic protein UN1952

>UN1952

CAAAAGTTAGTAACTACCCTCCTTGTCCTAAGCCAGAACTCATCAACCTTGTTGAAC
GAAAATGAAAAAAGTGAAGTTTATCCAAAATTTGTGTTTGATGATTACATGAAACTC
TACATGGGACTTAAGTTCCAAGCTAAGGAGCCTAGATTTGAAGCTATGATGAAAGC
CAGGACAAGTGTTGGTGTGGGACCATAATATTATTATTATTATTATTATGTTGAT
TATGCGTGTGGTTATATTATATTAATATGCTTTATTAATGTTTGGTTGGTGTTTAAGA
TTGTGCAGTCTAAAGGGTTATATTAAAAAATAAAGTAAGATTAATTAGTTTGATCTT
AGATTGTGTGTGAGAATCAGTTATGATTAACTGATTAAGTTTATGTAATGCGATGCA
GTGATTTAGAACTGTTTTTATAAAG

Unigene sequence of the polymorphic protein UN2594

>UN2594

TATAATCAAAGCAATTACATTTTTCTTCTTCTCAATTCAGATCAACTTAACACAAAAA
AAAAAACATAATAGCATCTATTAAAACATACATGATGGACAAGCAATTTCTCAACA
AAAAAAAAAAACTGTACATCCCCGAGAAATCTCAACCTTAACCTTCCCTTAAGAAA
AGGATTTAATTAAAATTGCCCTTAATTAACTGGGACCCCAGCAGCTTAGGTACATTA
CTGTCCTCAAAGACTCCTCTGGCTGTTTTGCTTTATCATCTTTTAGTCTTTTAGAAATG
ATAGCAGAAGAATTGGAAGAAGAAAGCTTATTTGCCTGAGAGAAAGATCTAACATT
GTTTTTAACATGTTGCTGAGCTGATTTCAAAGCATAATTCCATCTACAGATGCCTTGG
TCTTTCAAGGCCTCCACTACACCAACGCTAACAGCCACACTCCAAGCTCTGATTCCT
GAACTCATCAT

Unigene sequence of the polymorphic protein UN2787

>UN2787

AATGAATTTCTCCGCCGCTTCATCGTCGTTAATCGGAACTCAAACTAACAATTTCCAT
AACAATAAGCTACAAAAAGCGCGTTTGCGTTCATCTTCTTCTTCATTTTCACTTAACA
CCACTCACCACCACCACCACCACCACCGACAACCACTACACCTTCATTTTCGTTCTTC
CAATGTCCTTCCTCTCCGTTCATCCTATACGCGATCTCTTTCTCATTCTTCTATGACTG
TGAAGGCGGTGGCGAAGTCGGAGGAGCCGCTACGTGTTATGATTTCCGGTGCTCCTG
CTTCTGGTAAAGGAACTCAATGCCAGCTTATTGCTAACAAATATGATTTGGTGCATG
TTGCTGCTGGAGATTTACTTAGGGCCGAAATTGAAACCGGAAGTGAAAATGGAAAG
CGTGCAAAAGGATATATGGAGAAGGGACAGTTGGTCCCTGATGAAATAGTTGTCAT
GATGGTCAAGGATCGTCTCTTGCAGCCAGATTCTGTAGAGAATGGTTGGCTTTTGGA

TGGATATCCCAGGAGCTTATCACAGGCTACTGCACTTAAAGAATTAGGGTTTGAACC
TGATATTTTTATTCTTCTAGAGGTCTCTGAAGATATTCTTGTGGAGAGAGTAGTTGGA
AGGAGATTAGATCCTGTTACTGGGAAAATATATCATTTGAAGAATTCTCCTCCAGAA
ACAAGAGAAATTGCAGATAGGCTTACTCAACGTTTCGATGATACTGAAGAGAAGGT
AAAAGTTGCGATT

Unigene sequence of the polymorphic protein UN2827

>UN2827

CAATCAATGCTTAACTACCAAAGAAGCAGAAAGCACATTGCACATATTGCTGTGCAT
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CAAAAGAGAGC