DETERMINATION OF GENETIC VARIATIONS OF CHICKPEA, LENTIL AND ALMOND GENOTYPES STORED IN THE JORDANIAN GENE BANK USING PCR-BASED MOLECULAR MARKER

PhD Dissertation

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Eskişehir 2022

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Eskişehir

Eskişehir Technical University

Institute of Graduate Programs

November 2022

This thesis has been supported by the project 21DRP101, which is approved by the SRP Committee.

FINAL APPROVAL FOR THESIS

This thesis titled DETERMINATION OF GENETIC VARIATIONS OF CHICKPEA, LENTIL AND ALMOND GENOTYPES STORED IN THE JORDANIAN GENE BANK USING PCR-BASED MOLECULAR MARKERS has been prepared and submitted by Ghina AL-HMOUD in partial fulfillment of the requirements in "Eskişehir Technical University Directive on Graduate Education and Examination" for the Degree of PhD in Biology Department has been examined and approved on 03/11/2022.

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SUPERVISOR APPROVAL

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Supervisor

Prof. Emel SÖZEN

ABSTRACT

DETERMINATION OF GENETIC VARIATIONS OF CHICKPEA, LENTIL AND ALMOND GENOTYPES STORED IN THE JORDANIAN GENE BANK USING PCR-BASED MOLECULAR MARKERS

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The genetic diversity of some chickpea and lentil accessions collected from different regions adapted from the Jordanian Seed Genebank presented in NARC with some cultivated almond and local wild genotypes were determined using ISSR and SCoT markers. For chickpea genotypes the polymorphism average and PIC values obtained from ISSR and SCoT primers were 72% and 75%, 0.17 and 0.26, respectively. In STRUCTURE analysis, SCoT markers were found to be more informative than ISSR. For lentil genotypes the polymorphism average and PIC values in ISSR and SCoT primers were found to be 92% and 89%, 0.24 and 0.27, respectively, and high genetic similarity values were found between local and wild landraces demonstrated by the both markers. In STRUCTURE analysis, 2 different populations were obtained with ISSR markers and 3 different populations with SCoT markers. In almond genotypes the polymorphism average in ISSR and SCoT primers were 90 % and 93 % respectively, while the PIC value was 0.25 in both markers. The genetic similarity values between the cultivated and wild almond genotypes were high and almost the same for the two markers. The ISSR marker showed to be more informative than SCoT marker evaluated by STRUCTURE analysis which produced three populations instead of two.

Studies on genetic diversity for Jordanian plant species, especially legumes and their wild relatives, are very few, our study results have contributed to this area. The findings obtained from ISSR, and SCoT markers provided important data for the characterization of the samples inside the seed genebank.

Keywords: Plant genetic resources, Seed genebank, ISSR, SCoT, Genetic diversity

ÖZET

PCR TABANLI MOLEKÜLER MARKIRLAR KULLANILARAK ÜRDÜN GEN BANKASINDA SAKLANAN NOHUT, MERCİMEK VE BADEM GENOTİPLERİNDE GENETİK VARYASYONUN BELİRLENMESİ

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Ürdün'ün farklı bölgelerinden toplanıp NARC Ürdün Tohum Genbankasında koruma altına alınan bazı nohut ve mercimek aksesyonları ile bazı yerel badem genotipleri ve kültür çeşitlerinin sahip olduğu genetik çeşitlilik ISSR ve SCoT markırlar kullanarak tespit edilmiştir. Nohut genotipleri için ISSR ve SCoT primerlerinden elde edilen polimorfizm ortalaması ve PIC değerleri sırasıyla % 72 ve % 75, 0.17 ve 0.26'dır. STRUCTURE analizinde SCoT markırlar ISSR'den daha bilgilendirici bulunmuştur. Mercimek genotiplerinde ISSR ve SCoT primerlerindeki polimorfizm ortalaması ve PIC değerleri sırasıyla %92 ve %89, 0.24 ve 0.27 olarak bulunmuş, kültür ve yabani ırklar arasındaki yüksek genetik benzerlik değerleri iki markırla da gösterilmiştir. STRUCTURE analizinde ISSR markırlarıyla 2, SCoT markırlarıyla 3 farklı popülasyon elde edilmiştir. Badem genotiplerinde ise ISSR ve SCoT primerlerinde polimorfizm ortalaması sırasıyla % 90 ve % 93 iken, her iki belirteçte de PIC değeri 0.25 olarak hesaplanmıştır. Badem genotipleri arasındaki genetik benzerlik değerleri, iki markır için neredeyse aynı değerlerde bulunmuştur. ISSR markırlarıyla yapılan STRUCTURE analizi çalışılan çeşit ve genotipleri 3 gruba ayırmıştır, SCoT markırları ise çeşit ve yabani genotipleri ayıran 2 populasyon oluşturmuştur.

Ürdün'ün bitki türleri, özellikle de baklagiller ve yabani akrabaları için genetik çeşitlilik üzerine yapılan çalışmalar çok azdır, çalışma sonuçlarımız bu alanda katkıda bulunmuştur. ISSR ve SCoT markırlarından elde edilen bulgular tohum gen bankasındaki örneklerin karakterizasyonu için de önemli veriler sağlamıştır.

Anahtar Sözcükler: Bitki genetik kaynakları, Tohum gen bankası, ISSR, SCoT, Genetik çeşitlilik

ACKNOWLEDGEMENTS

First and foremost, I am extremely grateful to Allah for giving me this opportunity which provide me a good experience in science and life, and giving me strength and encouragement throughout all the challenging moments of completing this dissertation. I would like to express my deepest appreciation to my supervisor Prof. Dr. Emel Sözen for her invaluable advice, support and patience during my PhD study. Her enormous knowledge and plentiful experience have encouraged me in my academic research time and daily life. I would like to express my deepest gratitute to my family; Dad, Mum, sister and brothers for being supportive all years along and believing in me. I would like to thank my laboratory colleagues; Kevser, Nazlı, Mücahit, Emna, and my friends all over the planet, in addition to every one helped and tried to help me collecting the Jordanian plant samples used in this study.

Ghina AL-HMOUD

03/11/2022

STATEMENT OF COMPLIANCE WITH ETHICAL PRINCIPLES AND RULES

I hereby truthfully declare that this thesis is an original work prepared by me; that I have behaved in accordance with the scientific ethical principles and rules throughout the stages of preparation, data collection, analysis and presentation of my work; that I have cited the sources of all the data and information that could be obtained within the scope of this study, and included these sources in the references section; and that this study has been scanned for plagiarism with "scientific plagiarism detection program" used by Eskişehir Technical University, and that "it does not have any plagiarism" whatsoever. I also declare that, if a case contrary to my declaration is detected in my work at any time, I hereby express my consent to all the ethical and legal consequences that are involved.

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GLOSSARY OF SYMBOLS AND ABBREVIATIONS

°C	:	Degrees Celsius
µm	:	Micromolar
AFLP	:	Amplified Fragment Length Polymorphism
BC	:	Before Christ
bp	:	Base pairs
CTAB	:	Cetyltrimethylammonium bromide
dH_2O	:	Distilled water
DNA	:	Deoxyribonucleic acid
dNTP	:	Deoxynucleotide triphosphates
ECPGR	:	The European Cooperative Program for Plant Genetic Resources
EDTA	:	Ethylene diamine tetra acetate
FAO	:	Food and Agriculture Organization
ICARDA	:	International Center for Agricultural Research in the Dry Areas
ISSR	:	Inter Simple Sequence Repeats
KCL	:	Potassium Chloride
mg	:	Milligram
$MgCl_2$	:	Magnesium chloride
min	:	Minute
mix	:	Mixture
ml	:	Millilitre
mM	:	Millimolar
NaCl	:	Sodium chloride
NARC	:	National Agriculture Research Centre
NBPGR	:	The National Bureau of Plant Genetic Resources
ng	:	Nanogram
PCR	:	Polymerase Chain Reaction
PGRFA	:	Plant genetic resources for food and agriculture
PGRs	:	Plant genetic resources
PIC	:	Polymorphism information content

PVP	:	Polyvinylpyrrolidone
QTL	:	Genetic and qualitative trait loci
RAPD	:	Random amplified polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism
rpm	:	Round per minute
RSCN	:	Royal Society for Conservation of Nature
SCOT	:	Start Codon Targeted Polymorphism
Sec	:	Second
SSR	:	Simple Sequence Repeat
TBE	:	Tris-borate-EDTA
USA	:	United State of America
WRPIS	:	Western Regional Plant Introduction Station
µg	:	Microgram
µl	:	Microlitre

1. INTRODUCTION

1.1. General Introduction

Plants are the main source organism living on Earth. They supply food to all the organisms including humankind, in addition to their importance in maintaining the atmosphere with photosynthesis. Plant diversity changed with years, as Mankind travel from place to place for food and water resources as a major purpose or other reasons as volcanoes, earthquakes, or war. As each community has its traditions and culture; they used to plant and propagate their own food. The first cultivated plants were cereals and legumes as they were major food crops. Later, other species were used for their fruits or roots and were expanded and adapted up to their current ecological limits. Migration movements were the first means of transportation, followed by trade routes, which were often used to transport goods over great distances (Esquinas-Alcázar J. T., 1993).

In the past, our ancestors use several types of crop varieties and choose the best ones by their phenotypic characteristics, as the more producing, larger seeds size, pest resistance and frost resistance types. By those simple methods they select the best plant genetic resources until nowadays. Local genotypes and varieties were developed by thousands of years of selection by farmers and the nature, which were influenced by climate and many other environmental factors (Esquinas-Alcázar J. T., 1993). Animals, microorganisms, and invertebrates all contribute significantly to the genetic resources for food and agriculture, which are important for feeding humankind (Sonnino, 2017). The genetic diversity of plants is essential for human survival. By selecting the best plant genes that can survive in harsh conditions, they serve as both the base for agricultural development and a reservoir of genetic adaptability. This allows better plant varieties to be used and combined in a proper manner and provide an irreplaceable characteristic as local adaptation, resistance to disease, and productivity (Esquinas-Alcázar J. T., 1993).

Every country has its' special plant diversity due to its climate, weather, soil type in addition to other factors, as the Mediterranean basin have its unique plant varieties and genotypes that not presented in Europe or Tropical areas in Africa and *vice versa*. However, all humans share in the Earth's plant genetic resources, and our ability to eat depends on the long-term management of these various biological resources. These resources are essential for food production and agriculture (Halewood, Noriega, & Louafi, 2012). To conserve the inherent genetic variability used in breeding programs, genetic resources must be preserved (Cunha Alves & Azevedo, 2018). For over 500 years,

plant genetic resources for food and agriculture (PGRFA) have been regularly gathered and exchanged (http-1).

All countries tried to conserve their plant diversity and genetic resources by making exceptional areas or gardens, collecting plant samples as dry samples (herbarium) or collecting plant seeds placing them in special location called seed bank, as a long-term preserving resource, which needs a huge financial support from qualified authorities (Fowler & Hodgkin, 2004). Plant genetic resources (PGRs) are important in handling the global challenges that are currently facing the human population, especially climate change and food scarcity (Wambugu, Ndjiondjop, & Henry, 2018), and the main reasons for conserving plant genetic resources for food and agriculture (PGRFA) are to ensure the future adaptability of cultivars and wild populations, to preserve genetic variety for cultural reasons, to ensure the preservation of data and traits that ensure sustainable agriculture, to encourage the use of genetic resources in commercial and biotechnology (http-1).

Agricultural PGRs include wild relatives of domesticated species, varieties, and hybrids, along with breeding stock, ornamental, medicinal, and also other plants that can be developed for use in agriculture and serve as a source of food for both living creatures (Bošković, et al., 2012). However, to preserve plant genetic resources, genebanks' long-term aim as an agricultural heritage for enhancing crops in the future. Plant propagation techniques and seed storage have been practiced for decades. 7.4 million germplasms are saved and conserved in some more than 1750 genebanks globally (Mascher, et al., 2019), they are function as a significant source for genetic material in genomic research in a variety of plant science fields (Wambugu, Ndjiondjop, & Henry, 2018). Stored seeds in genebanks can be considered historic documents that provide information about the history of agriculture (Mascher, et al., 2019). Genebanks distribute and protected more than 16,500 plant species in different genebanks world widely (Sonnino, 2017).

The relative loss of genetic diversity in agricultural plants when genetically similar cultivars created by regular breeding began to overtake traditional landraces world widely inspired the creation of large *ex-situ* collections of conserved plant genetic resources (Mascher, et al., 2019). Those large numbers of genebank collections have some challenges such as unnecessary duplicates within and between genebanks. Genetic data for all accessions stored in genebanks will help us avoid this problem and will make it easier for researchers, breeders, and institutional users to obtain germplasm. Some

germplasms have distinctive genotypes that were kept as growing plants, assessed for their phenotypic and genotypic properties, documented in a national public germplasm database, and given away for free as clonal propagules to researchers and other germplasm users around the globe (Postman, et al., 2006). A reliable genetic data set serves as a continuous resource for genotype-phenotype mapping (Mascher, et al., 2019).

Germplasm conservation includes crucial elements like germplasm characterisation and evaluation. Without adequate characterization and evaluation, considerable genetic variety in the collections cannot be used successfully for plant progression. Genetic markers have a lot of potential for use in crop development in addition to their utility in characterizing genetic resources. Genebank managers and conservationists have long been concerned with making sure that the preservation of genetic diversity, whether *in situ* or *ex situ*, covers a sizable portion of the genetic data on the germplasm they work with.

PCR based molecular markers are useful to estimate genetic diversity of collections within and between population levels to determine divergent subpopulations that potentially contain valuable genetic variation. In addition, they can help us distinguish any duplicates and organize collections. Additionally, markers can identify useful genes that are present in collections. A core collection that accurately represents the complete collection can also developed using molecular marker data.

The primary objectives of this study were:

1- To determinate the genetic variation of different chickpea, lentil and almond accessions deposited in the National Agriculture Research Centre (NARC) of Jordan using ISSR and SCoT markers.

2- To compare genetic diversity levels of wild lentil and almond landraces with adopted ones and investigate the relative genetic contribution between local cultivated landraces and some of its local wild landraces in the case of lentil and almond accessions.

3- To find out any possible duplication of accessions presented in the seed genebank after identifying chickpea and lentil genotypes.

1.2. Plant Biodiversity of Jordan

Jordan is a small country with an area of 89,342 km^2 which lies between latitudes 29° 30' and 32° 31' in the Eastern Mediterranean. The country is divided into four separate biogeographical regions: the Mediterranean, Irano-Turanian, Saharo-Arabic (Badia), and

Sudanian (Tropical). Since Jordan has a variety of climates and topographies, its biological habitats and flora are very diverse (Syouf & Duwayri, 1996). Despite Jordan's small area, it is considered as a country rich in plant diversity compared to larger countries in the region such as Saudi Arabia and Egypt (Ayasra & Al-Mimi, 2021), When compared to the total number of vascular plant species that have been recorded on earth (250.000 - 300.000), Jordan's flora is abundant and diversified as about 2600 species in 114 families and 907 genera can be found (Brehm, et al., 2016), which represents 1% of the overall world flora (Brehm, et al., 2016; Ayasra & Al-Mimi, 2021). There are 106 endemic and near-endemic species in the Jordanian flora as reported in Brehm, et al. (2016).

This diversity is primarily caused by the significant topographical and climatic variation, which results in a large variety of ecological habitats and microhabitats. In addition to the four biogeographical regions previously mentioned, the vegetation cover map of Jordan which was developed by Prof. Dr. Dawood Al-Eisawi from 1985 to 1996 (Ayasra & Al-Mimi, 2021) includes thirteen vegetation types named as; the Acacia and rocky vegetation, ever-green Oak Forest, deciduous Oak Forest, Juniper Forest, Mediterranean non-forest vegetation, Hammada vegetation, Pine Forest, sandy dunes, steppe vegetation, tropical vegetation, halophytic vegetation, hydrophytic vegetation and mud flats. As a result, the Jordanian flora genetically and ecologically adapted to local conditions, and most species are even suited to the country's arid regions. Over only 100 km distance; altitude ranges from 400 m under the sea level (dead sea) up to 1750 m at Jabal Rum, the climate ranges from semi-arid conditions with less than 200 mm of rainfall to semi-humid Mediterranean conditions with more than 500 mm of rainfall (Brehm, et al., 2016).

The concept of important plant biodiversity areas has developed to be part of a global program that includes the main biodiversity areas (Areas Biodiversity Key) which contributed significantly to the global stability of biodiversity (Ayasra & Al-Mimi, 2021). In 2003, the first important plant area defined in Turkey. After that the initiatives continued, and plant areas identified in more than 27 countries including North Africa, Europe, and the Middle East. The status and importance of these sites demonstrated by integrating them into the national plans for the preservation of plant diversity, as in some countries these sites became protected areas under the law. The south areas (Al-Qadisiyah Mountains/Dana) of the country that contained juniper forests in 1800’s, decreased dramatically while making the Hejaz railway, in addition to many sites along the railway

between 1900-1908. The environmental concern within Jordan has begun since the establishment of the Emirate of East Jordan, when the first forest protection law was issued in 1922. The number of natural reserves has reached at the present time to 12 nature reserves in various regions of Jordan which forms 5% of the area (Ayasra, and Al-Mimi, 2021), while the goal was to reach 12% as protected areas of Jordan's overall area in 2012 and to achieve 15% in 2017 (Al-Fyad, et al., 2007).

Jordan is located at the south-western end of the Fertile Crescent of the Middle East, which is the birthplace of temperate agriculture and where the Vavilov centers of Asia Minor and the Mediterranean share a border. The center of Asia Minor is the crop origin for wheats, barley, rye, oat, lentil, and chickpea, in addition to several forages, oil-producing plants, vegetables, melons, fruit crops and dye plants. A significant diversity of landraces and wild relatives of these crops can be found in Jordan. Over the past 10,000 years, more than 14 crops with worldwide significance have been domesticated in the area and have gradually spread throughout the world. A recent study of global patterns of crop wild relatives diversity that include 108 highest priority genera has found in the Fertile Crescent which contains 254 special taxa; therefore, it is the region with the highest concentrated crop wild relatives globally per unit area, and its resources of agricultural diversity is the key to maintain of global food security (Brehm, et al., 2016).

Plant diversity in Jordan is facing a dramatic decline because of habitat loss, degradation and to a considerable risk of extinction (Al-Fyad, et al., 2007). Many factors threaten the wild plant diversity, as increasing development pressures combined with population growth, tourism development (increasing number of visitors, litter accumulation, effluent discharge, and extensive water extraction, encroachment by local population, roads development and including intensive use of resources), climate change, uncontrolled vehicle movement, overgrazing and extensive woodcutting, uncontrolled urban expansion (including transformation of forests into agricultural and urbanized areas), intensive and extensive agricultural practices, unbalanced water use and unplanned water extraction, unplanned mining and quarrying, introduction and spread of invasive species, weak enforcement of law, and lastly refugees pressures that related mostly to the regional political crisis (including pressures on water resources, agricultural areas, rangelands and open spaces, as well as ecosystem goods and services) (Brehm, et al., 2016).

1.3. Plant Genetic Resources

Humankind has been supported for several millennia by the plant genetic resources forming the natural variations. These resources are the foundation for food security, energy, animal feed, fiber in addition to other ecosystem services (Wambugu, Ndjiondjop, & Henry, 2018). With the human movements, as well as food crops traveled out of their original native environments with the occasional help of birds, winds, and ocean currents. Since the early Neolithic eras, agricultural crops and the genetic resources that go along with them have been dispersing around the world (Fowler & Hodgkin, 2004).

Humanity's survival depends on crops; to meet the expanding human needs, they must become increasingly more productive, through genetic gains that exploit diverse plant genetic resources (PGRs) that have been conserved by individuals, communities, and organizations (Bretting, 2018). These genes have been distributed throughout regional cultivars and wild plant populations over thousands of years by farmers and nature for their adaptability, resistance, and production traits. The modern phenomena of agriculture started when people began to domesticate wild plants for their nutritional value. Numerous cultivars suited to their particular conditions were produced by this evolutionary process, making up a vast genetic resource (Esquinas-Alcázar J. T., 1993).

PGRs is a key component of the global genetic commons is agricultural biodiversity (Halewood, Noriega, & Louafi, 2012). PGRs will continue to be in the future, independently valuable for scientists whether for conventional plant breeding or modern genetic engineering (Esquinas-Alcázar J. T., 1993). For plant breeding and agricultural production, genetic resources strengthened both of them, thus they are essential to food security, livelihoods, and the development goals on Earth (Fowler & Hodgkin, 2004). The original hosts of PGRs were farmers, gardeners, families, and agricultural communities. Now a days they also include institutions, organizations, networks, and governments (Bretting, 2018).

The conflict for access to useful plants for agriculture and food coming from other regions has been one of mankind's primary reasons for travel since early times, and the history of genetic exchange resources is the history of mankind (Esquinas-Alcázar, Hilmi, & Noriega, 2012). Several European powers in the colonial period had the marine and military technology to locate and move plants around the world in an organized manner,

with the capacity to test, develop and supply them to distant production sites through networks of botanic gardens (Fowler & Hodgkin, 2004).

Landraces are important genetic resources that improve gene pools of modern cultivars through introducing new alleles with its specific genetic background which can be used in genetic research programs (Pakseresht, Talebi, & Karami, 2013). The rapid expansion of plant breeding during the second half of the 20th century leads the introduction of a huge number of improved varieties, which gradually replaced old landraces, especially in developing countries. The release of commercial varieties drives to decrease the number of varieties cultivated in traditional farming systems (Sonnino, 2017). Since 1950s, the Food and Agriculture Organization (FAO) of the United Nations, have had serious concerns over the loss of the genetic resource's diversity in the world (Esquinas-Alcázar, Hilmi, & Noriega, 2012).

In 1974, The International Board for Plant Genetic Resources (The International Plant Genetic Resources Institute now) has been established and originally housed in the FAO, assumed the mandate for catalyzing and coordinating a global initiative to collect and conserve Plant Genetic Resources for Food and Agriculture (Fowler & Hodgkin, 2004). The International Treaty of Plant Genetic Resources for Food and Agriculture (PGRFA)was established in the early 2000, to conserve and utilize plant genetic resources (Fu, 2017; Aubry, 2019). The adoption of this International Treaty has provided a regular framework to exchange, access and benefit sharing of PGRs (Sonnino, 2017).

The stakeholders of PGRFA specified a major restriction in the usage of conserved germplasm, the difficulty to access and obtaining associated information. PGR accessions are only available if global information databases are present and being updated; this requires collecting data about accessions from many collections into a single source, which allows it to transfer germplasm across institutions. Due to this, PGRs preservation and utilization is a great priority on improving information methods that link collections together to establish a single searchable database of genetic resources. (Guzzon & Ardenghi, 2018).

1.4. Plant Genetic Resources in Jordan

Jordan considered a center of origin of wheat, barley, food, and forage legumes and many fruit trees such as almond and figs. A significant diversity can be found in the country among cultivated landraces of grapes, olives, wheat, barley, chickpea, and lentils. In addition to those crops, the products derived from them such as olive oil, olive pomace,

wheat dough, burgul, and freeka, play a central role for the food security and livelihood of Jordanian farmers. Some crop species are now considered to be on the edge of national extinction (Al-Fyad, et al., 2007). A national and worldwide legacy, Jordan's PGRs are a part of the Fertile Crescent, and they should be protected and used for the benefit of humanity (Syouf & Duwayri, 1996).

Given Jordan's location in a region with unstable political conditions, the country's climatic conditions, and its rapid urbanization and development, which have caused habitat fragmentation and ecosystem degradation, it has given conservation and sustainable use of biodiversity components a high priority. The Forests Law, the first national law on biodiversity conservation, was adopted early in 1927, making conservation a cornerstone of governmental policy. With the conformation in 1993 of the Convention on Biological Diversity, the National Agriculture Research Centre (NARC), which is in charge of research and biodiversity conservation under the Ministry of Agriculture, presides the conservation and sustainable utilization of Plant genetic Resources for Food and Agriculture (RGRFA) and is formally assigned in 2001 as the centric point of the International Treaty of PGRFA (Al-Fyad, et al., 2007).

The country has established a genetic resources unit in NARC in 1993, however before that, most of collected materials were preserved mainly in ICARDA genebank, or International Center for Agricultural Research in the Dry Areas genebank. Germplasm of food legumes mostly lentils, chickpeas, and their wild relatives were collected from Jordan and stored at ICARDA (Syouf and Duwayri, 1996). ICARDA was established in 1977, with a head office in Aleppo-Syria, to support agricultural development in the dry areas of developing countries. ICARDA’s guardianship crops are barley, wheat, food legumes (chickpea, lentil, and faba bean) and feed legumes (vetch and grass pea). Its genebank holds over 154,000 accessions from over 110 countries (Reddy, 2017).

There was a concept that has been addressed as “On-farm management”, which was applied by NARC from 1999 to 2005. The project supports on-farm conservation concept of agro-biodiversity which train the farmers on mechanisms and techniques on management of the biodiversity within the farm level, thus helped the farmers to establish their field genebanks and train them on their management. The Royal Society for Conservation of Nature (RSCN) managed the conservation and sustainable use through the full participation of local community in planning, application and management of the reserve and use its biodiversity in sustainable way (Al-Fyad, et al., 2007).

1.5. Genetic Diversity

Genetic diversity can be theorized as the key factor for population survival and evolution (González, Gómez-Silva, Ramírez, & Fontúrbel, 2020), and it is the sum of genetic characteristics within any species or genus (Rauf, da Silva, Khan, & Naveed, 2010). The estimation of genetic diversity is essential for crop improvement, efficient management, and conservation of germplasm resources and although within the parents it is necessary to improve the chances of selecting better segregates for various characters (Tahir & Karim, 2011). Genetic diversity helps maintaining ecosystem functions, stability, and has the same impacts on community structure and ecosystem processes as species diversity (Hoban, et al., 2020). Plant breeders focusing on the selection of plant type better suited to human needs, that outcome to more uniform genotypes in growth and reproduction which were selected and released for general cultivation (Rauf, da Silva, Khan, & Naveed, 2010) and this will lowering the genetic diversity driving to increase extinction risk (Hoban et al., 2020).

The estimation of genetic diversity can be different, which include morphological traits, biochemical or molecular markers (Pakseresht, Talebi, & Karami, 2013). Improving genetic diversity plays a significant role in pest and disease management and provides chances for further species advancement. Applied aspects as molecular markers-based genetic diversity are necessary for genetic mapping and marker-assisted selection in breeding. Many factors' effects on the reduction of genetic diversity of crop species such as urbanization, and the replacement of traditional agriculture systems by modern industrial methods (Rauf et al., 2010), so this reduction of genetic diversity can be noxious to a population as limiting its adaptation capabilities (González, Gómez-Silva, Ramírez, & Fontúrbel, 2020). On the other hand, few reports have indicated that modern plant breeding methods and systems also adjust the bulk of genetic diversity (Rauf, da Silva, Khan, & Naveed, 2010).

Genetic diversity knowledge is a valuable tool in genebank management and breeding experiments as in elimination of duplicates in the genebank stock, tagging of germplasm, and establishment of core collections (Tahir & Karim, 2011). Choudhary, Khanna, & Jain (2012) mentioned that "the knowledge and management of the genetic diversity and relationship within and between the cultivated chickpea and its wild relatives is essential and may ensure the long-term success of chickpea improvement programs". For a high genetic diversity within some populations, and with an effective

strategy for preserving genetic variation is to conserve many individuals for more diverse populations (Fikiru, Tesfaye, & Bekele, 2007).

Several types of molecular markers have been used to estimate the genetic diversity in crop species, and the choice of the technique depends on the objective of the study, sensitivity level of the markers, financial limitations, skills, and facilities availability (Fikiru, Tesfaye, & Bekele, 2007). However, DNA techniques allow unlimited numbers of polymorphic marker loci evaluation (Nguyen, Taylor, Redden, & Ford, 2004) The appearance of different molecular techniques led breeders to assess genetic diversity based on data generated by different molecular markers, which provided a means of rapid analysis of germplasm and genetic diversity estimation, which were often found to confirm phenotypic data (Rauf, da Silva, Khan, & Naveed, 2010).

1.6. Loss of Genetic Diversity (Genetic Erosion)

In the biggening of the 1900's it was been noticed that traditional crop varieties and highly diverse landraces were being lost from cultivated fields around the world, as uniform modern varieties began to replace them, which led to the loss of genetic diversity in a process known as "genetic erosion" (Henry, 2006). This term was created to explain this loss of specific genes and gene combinations at the Technical Conference Plant Exploration, Utilization and Conservation of Plant Genetic Resources of the FAO/IBP (International Biological Program), which took place in Rome in 1967. In addition to other factors including the appearance of new pests, weeds, and diseases, habitat degradation, urbanization, land clearing due to deforestation, and bush fires, the primary cause of genetic erosion and loss of biodiversity is the replacement of local types by newer ones. Loss of genetic diversity was found to be a contributing factor to crop vulnerability to pests, diseases, and other adverse effects following the outbreaks of corn-leaf blight and coffee rust, respectively, in the USA and Brazil (Sonnino, 2017).

Over the last 50 years, the dangers of genetic erosion in PGRs have realized and stimulated political and scientific movements around the world to conserve plant genetic resources (Fu, 2017). The erosion of PGRs threatens world food security. Thus, the emergence of new technology, as well as the substitution of native varieties with imported ones, the settlement of new territories, adjustments to farming practices, and other factors, led to a quick and drastic loss of plant genetics. The degradation of PGRs may result in the extinction of important species since it impacts both domesticated species and numerous wild species with direct or indirect agricultural uses (Esquinas-Alcázar J. T.,

1993). In the last few decades, genetic erosion in the form of alleles, genotypes, as well as domestic crop accessions has been observed as more than 70 % in many areas of the world (Guzzon & Ardenghi, 2018).

Jordan experienced a severe decline in plant diversity, which led to habitat loss and degradation, the isolation of many species, a loss of genetic diversity, and a significant risk of extinction. There are between 100 and 150 threatened and 200 to 250 rare plant species nationally (Al-Fyad, et al., 2007). Genetic erosion was observed in many cultivated species and one of the reasons is loss of local populations and their wild relatives, that is related mainly to the loss of habitat due to land use for agriculture, industrialization, and urbanization. In addition to human activities that have caused pollution of water, soil, and air, many plant and animal species have also been wiped off, which has had a major negative impact on the global economy. In Mexico, only 20% of the local maize varieties that were known in 1930 still exist today. In northern Thailand, the genetic diversity in traditional upland rice germplasm has decreased as a result of the replacement of many traditional varieties with fewer modern varieties. In China, there were about 10,000 varieties of wheat grown in 1949, but only 1000 varieties presented in 1970 (Bošković, et al., 2012).

Crop wild relatives contain a wealth of genetically important traits due to their adaptation to a diverse range of habitats and the fact that they have not passed through the genetic bottlenecks of domestication, in addition they host genetic diversity tested by natural selection in a range of environments. Plant breeders recognized the potential value of landraces since at least the early 20th century, however now with the advent of modern genomics combined with systematic phenotyping, it is feasible to revisit wild accessions and landraces to prioritize their use for specific agricultural purposes as disease resistance, drought tolerance, and nutrient density (Coyne, et al., 2020).

1.7. Conservation of Plant Genetic Resources

The importance of the conservation of genetic resources was recognized early as Sonnino, (2017) referred in his paper with the following examples: China maintained control of genetic resources that were considered crucial to maintain the silk production monopoly, but they sadly lost it in 550 AD when two Nestorian monks escaped with mulberry seeds and silkworm eggs into the Byzantine Empire for Emperor Justinian I. In 795 AD Charlemagne gave instructions through the decree "Capitulare de villis" to plant 77 different food plant species in each farm. The Venetian Senate also made an early

attempt to regulate the use of plant genetic resources in 1414 AD when it reported that the widespread use of cypress trees in Crete was putting them in danger of extinction. The Brazilian government forbade the export of rubber trees or seeds in order to maintain its monopoly on the production of rubber, but it was broken in 1876 when Henry Wickham smuggled a large quantity of seeds and began rubber production in British colonies such as Ceylon and Malaysia (Sonnino, 2017).

Before 1900, breeders began to assemble working collections of genetic resources, and as commercial agriculture spread, concern with the loss of traditional landraces led to calls for conservation (Fowler & Hodgkin, 2004). Before 1980, fruit and nut germplasm collections were primarily kept up to date in the United States by solitary plant breeders at universities. These collections, however, were sometimes destroyed as scientists retired, shifted the focus of their study, or ran into financial difficulties. As a result, collections made of valuable items that took years to put together frequently went missing (Postman, et al., 2006). If conservation strategies are not applied for *Triticum dicoccoides* which is the direct progenitor of durum wheat, it will be one of the most threatened species in Jordan. In addition to other crops such as the historical olive trees and wild almond are under threat due to the replacement of these species by the new varieties (Al-Fyad, et al., 2007).

Conservation efforts of plant germplasms are rooted in the realization of genetic erosion of crop genetic resources on agricultural farms introduced by modern plant breeding (Fu, 2017). The need for worldwide regulation and organization of the conservation and utilization of genetic resources has only recently become apparent due to scientific advancements in germplasm discovery and the economic interests stimulated by germplasm exploitation. Additionally, the conserved germplasm offers a wide genetic diversity that is used more often for genetic research and plant breeding programs, undoubtedly having a positive impact on the current and future global food production (Sonnino, 2017). The goal of conservation is to empower sustainable development by protecting and using biological resources without compromising the wealth of species and genes (Bošković, et al., 2012).

Though, plant conservation genetics, along with most other areas of biology, has been progressed by developments in molecular biology. The power of molecular genetic analysis and the increasing availability of cost-effective technologies permit plant conservation genetics to be applied much more widely in support of plant biodiversity

conservation (Henry, 2006). The provider of our future conserve PGRs are the organs and tissues (seeds, fruits, cuttings, pollen, tissue cultures, etc.), by which plants can be propagated (Bretting, 2018). As FAO mentioned in its website (Food and Agriculture Organization (FAO), n.d); "PGRs can be conserved both *in-situ* and *ex-situ*. *In-situ* conservation (on-farm conservation) means the maintenance and recovery of viable populations of species in their natural surroundings. While *ex-situ* conservation means conservation of biological diversity components outside their natural habitats and the main conservation techniques are genebanks". Henry, (2006) mentioned in his book that "the conservation of plants *in-situ* is the main option for plant with little value as ornamentals, in agriculture or forestry, while *ex-situ* conservation is largely employed for economic species, especially those used in agriculture. However, the conservation of many economic species can be enhanced by conservation of wild relatives *in-situ*".

In-situ conservation can be considered as a very sensitive method that can be endangered by fires, extreme weather conditions, and other factors; while *ex-situ* conservation methods include seed storage, field genebank, DNA storage, pollen storage, *in vitro* conservation, botanical gardens, cryo-conservation, and molecular marker technology. *Ex-situ* conservation of plant germplasm, especially seed banking, is a widely used method for the conservation of PGRs (Guzzon & Ardenghi, 2018), as seed storage is one of the simplest methods for long-term preservation of plant genetic material (Bošković, et al., 2012) that is fundamental for food security and plant breeding to hold off the losses in agrobiodiversity and to meet the global challenges (Guzzon & Ardenghi, 2018).

While the botanical gardens have traditionally focused on wild species that are endangered in their natural habitat, which specialized in ornamentals, medicinal and aromatic plants, and tropical species as some of these botanical gardens had a seed storage facility (Henry, 2006). Ex-situ conservation has had more success than *in-situ* conservation, probably because of its lower cost (around 100 times less than *in-situ* conservation) and major ease for users to access the material (Díez, et al., 2018). We can say that long term plant *ex-situ* conservation can prevent moreover genetic erosion, even plant species extinction (Henry, 2006).

Ex-situ field gene banks for fruit tree landraces and their wild relatives were created at various research facilities in Jordan. NARC in Jordan identified several hot spots with high priority for *in-situ* conservation of wild relatives and landraces for specific crops',

as the agro-biodiversity *in-situ* sites in Ajlun province. The Conservation of Medicinal Herbal Plants project found at least 10 more *in-situ* conservation sites were identified and managed in 2007 (Al-Fyad, et al., 2007).

1.8. Genebanks

A genebank, also known as a seed genebank, is a repository where seeds or germplasm that are not widely used economically and are in danger of extinction are stored and disseminated. To ensure that samples of various crops do not completely vanish, seed banks were established. It is comparable to a savings account where seeds are deposited with the aim of withdrawing from them in the future as needed. The seeds can remain viable for decades or even centuries if they are properly stored, maintained, and protected (Reddy, 2017). They were created to secure the survival of gene combinations when realized that the biodiversity of crops had stopped increasing and started to decrease (Fu, 2017; Díez, et al., 2018).

Genebanks were created in the middle of the twentieth century to preserve cultivated biodiversity while landraces began to be replaced by modern varieties and this step was accepted as a necessity for future protection (Díez, et al., 2018). After the establishment of The International Board for Plant Genetic Resources in 1974, to hold off the loss of plant diversity; collecting missions were accelerated and genebanks were expanded at national, regional, and international levels (Henry, 2006). Genetic materials for genebank collections are obtained from *in-situ* conditions, breeding, and research projects. Sadly, a lot of these materials can no longer be located *in-situ* since they have either been abandoned or replaced by modern cultivars (Fowler & Hodgkin, 2004).

Genebank represents the most cost-effective *ex-situ* conservation strategy (Fu, 2017) by providing a suitable source of materials for most institutional users, breeders, and researchers, containing large numbers of accessions coming from many different places (Fowler & Hodgkin, 2004). Therefore, genebanks were never meant to serve a pure preservation records of crop diversity unless had the future looking aim of preserving the evolutionary potential of crops (Mascher, et al., 2019) that to preserve genetic material, either directly or as data in breeding programs to face potential changes in environmental conditions or societal needs (Díez, et al., 2018). The number of *ex-situ* genebank storage facilities increased rapidly from 54 genebank at the end of 1970's to almost 1.470 genebank in 2002, conserving more than 5.4 million accessions, which they are mainly PGRs for food and agriculture (Henry, 2006).

Seeds are the basic unit in the whole plant, so seed banking was the principle method used for conserving species *ex-situ*, in addition to the most researched, efficient and effective form (Henry, 2006) which shows several advantages as a long-term *ex-situ* conservation strategy as; it is relatively easy to collect, can be stored in small spaces, provide a decent sample of the genetic diversity within the species, remains viable for long periods, in addition to seed collections do not require a high level of maintenance and are also economically viable (Guzzon & Ardenghi, 2018). Seed genebanks had been established in large numbers world widely and plant germplasm has been saved and protected. In this regard, seeds are readily accessible in 1,700 genebanks throughout the world (Reddy, 2017).

Whoever, seeds are vulnerable and endangered to a variety of threats; climate change and extreme weather conditions cause new pests and diseases, resulting in the extinction of certain species of plants, such as the natural disaster 'tsunami' in 2004, destroyed the rice paddies in Malaysia and Sri Lanka; the robbery of Iraq's essential seedbanks during the Iraq War (started in 2003) is an example of man-made disasters and during that War the plant scientists, fearing for the future of more than 1,000 seed varieties (everything from ancient wheat to chickpeas, lentils, and fruits) shipped them off safer place to ICARDA in Aleppo-Syria (Reddy, 2017).

It is usual for food species to be extinct throughout the world for a several reasons. From the 7,000 apple varieties in the United States in the 1800s, fewer than 100 varieties remain today and from the thousands of varieties of rice in the Philippines, only about 100 are grown now, while in China, 90 % of the wheat varieties that were cultivated a century ago are now extinct (Reddy, 2017).

The genebank has replaced the botanic garden, as it is the principal source of materials and collecting from the wild (Fowler & Hodgkin, 2004). Since the first plant genebanks were formed, enormous numbers of genes and the gene combinations produced by evolution have been stored in genebanks. Therefore, the primary purposes of genebanks are collection, documenting, regeneration, distribution, and conservation. Breeders now have additional tools available to produce variety and new data sources. Our planet has a variety of habitats, that in each habitat the maximum productive efficiency for each species is achieved with specified gene combinations. Therefore, many of these genotypes and their ancestors are certainly conserved in genebanks (Díez, et al., 2018).

In Jordan since 1995, landraces conservation activities had begun. The biodiversity program at NARC collected and conserved about 100 vegetable landraces in the genebank. The diversity of wild, semi-domesticated and underdeveloped species also plays a necessary role to ensure food security of rural people, considering that more than 100 edible-wild plants are utilized by local communities in Jordan. Several projects and activities for *in-situ* and *ex-situ* management of PGRs, have been implemented. The national genebank for *ex-situ* conservation of NARC stored and documented more than 3,200 accessions from 300 species makes them available for utilization purposes (Al-Fyad, et al., 2007). Recently NARC's national genebank, hosts more than 4,000 seed accessions of different plant groups, including crops and landraces.

A seed genebank can hold several accessions for each crop. Numerous quantitative and qualitative data can be recorded during their characterization and evaluation, and typically a large intra accession diversity is seen (Laghetti, Pignone, & Sonnante, 2008). Because maintaining more accessions in a genebank costs more money and staff, there is always a limit to the number of accessions that can be conserved, analyzed, and used (Saad & Rao, 2001). There are some challenges with large genebank collections, like unnecessary duplications inside and between genebanks. Genetic data for every accession kept in the genebanks is required to avoid this difficulty. However, the management of all the information's becomes difficult without effective statistical methods (Laghetti, Pignone, & Sonnante, 2008).

1.9. Chickpea and Lentil Plants

Chickpeas and lentils are a part of the second big family Fabaceae (or Leguminosae), which comes the second after the Gramineae family in their importance to humans. primarily distinguished by their distinctive floral structure, podded fruit, and 88% of the species' capacity to create rhizobial nodules (Duke, 2012). Legumes have a wide geographic distribution, a wide range of morphological, physiological, and chemical variety, and have played a crucial role in the evolution of humans. They are crucial elements of all agricultural systems around the world (Singh, Chung, & Nelson, 2007), between 12 and 15% of the Earth's surface is arable farmland, as they grow. They produce 27% of the principal crops grown worldwide, with grains and legumes alone meeting 33% of the world's population's dietary protein and nitrogen needs (Duke, 2012). Additionally, they are a great source of timber, drugs, tannins, gums, insecticides, fish poisons or

molluscicides, resins, vanishers, paints, dyes, and lacquers (Singh, Chung, & Nelson, 2007).

1.9.1. Chickpea

1.9.1.1. Chickpea history and distribution

The chickpea, *Cicer arietinum* L., was first domesticated at least 9500 years ago in the Fertile Crescent, which extends from Turkey to Iran, and at least 4000 years in the Indian subcontinent (Redden & Berger, 2007). Chickpea was domesticated as a crop around 10,000 years ago in Southwest Asia (Anatolia), then spread throughout the Fertile Crescent, to South Asia, East Africa (Ethiopia), Australia, and to North America (Sani, et al., 2018).

One of the earliest grain legumes grown by modern people, chickpeas were first cultivated and consumed in Turkey and Syria in the eighth millennium BC, according to archaeological evidence at Tell El-Kerkh and Tell Abu Hureyra in Syria; 7500–6800 BC at Çayönü; 5450 BC at Hacilar in Turkey; and recorded in Egypt and the Middle East as early as 3300 BC. It was introduced to the Americas in the sixteenth century AD by the Spanish and Portuguese. The Kabuli chickpea type was migrated through the Silk Road to India in the 18th century from the Mediterranean, whereas Indian immigrants to Kenya in the later 19th century brought the Desi chickpea type. Lately chickpea breeding programmers began in the USA, Australia, and Canada (Redden & Berger, 2007).

Cultivated of *C. arietinum*, is part of a group of annual chickpea species native to Mediterranean regions, while the majority of the remaining species in the genus are perennial species native to colder climates in Anatolia, the Caucasus, and Central Asia (Coyne, et al., 2020). Chickpea was developed in combination with other crops as wheat, rye, barley, lentil, peas, vetch, and flax as a part of the Fertile Crescent's agricultural development long years ago, from western Iran via Iraq, Jordan, and Palestine to Southeast of Turkey, a balanced arrangement of domesticates was developed which met all the necessities for humanity from carbohydrate, protein, oil, vegetable and animal fiber for rope and clothing (Redden & Berger, 2007).

In the present day, about two-thirds of the world's chickpea crop is grown in the Mediterranean, North Africa, the Middle East, and India, where it became widely cultivated (Redden & Berger, 2007). t came in third in importance among cool-season dietary globally, after dry bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.).

Currently, it is grown in about 33 nations and areas, including Australia, Central-West Asia, South Europe, Ethiopia, and North Africa (Aggarwal, et al., 2015).

1.9.1.2. Chickpea description

C. arietinum is a cool season grain legume (Saeed, et al., 2011), as Linnaeus describe it in1753; 12–50 cm tall, erect annual. imparipinnate leaves with oblong-obovate to elliptic leaflets, 25–75 mm length, stipules triangular to ovate shown in Fig. 1.1, corolla white, pink, purple, or pale blue; racemes 1–2 flowered. Large, globular or oblong, smooth or rough seeds, never reticulate, with variable range in color (Redden and Berger, 2007). A self-pollinated legume species (2n = 16), typically grown in the semi-arid regions (De Giovanni, et al., 2017).

Figure 1.1. *Cicer arietinum, The Photos copied from Kew Royal Botanic Gardens, England*.

Two commercial types of chickpeas are grown globally, differing in their morphological features: desi and kabuli types shown in Fig. 1.2; the desi types (microsperma) are recognized by their black seed coat and angular seed shape (De Giovanni, et al., 2017), With pink flowers, a stem that is anthocyanin-pigmented, rough seeds, and a growth habit that is either semi-erect or semi-spreading (Singh, Sharma, Varshney, Sharma, & Singh, 2008), has small seeds, leaves, pods, and stature which are mainly produced in Asia (Iran and India, certain parts of Turkey, but central Asia not included) and Ethiopia. While the kabuli types (macrosperma) are characterized by large leaves, pods, and seeds as well as a higher stature (Redden & Berger, 2007), white flowers, the seeds are lack of anthocyanin pigment, smooth, beige-colored, owl-shaped

seeds, with a semi-spreading growth habit (Singh, Sharma, Varshney, Sharma, & Singh, 2008), generally grown in Mexico and in the Mediterranean region (De Giovanni, et al., 2017) including Western Asia, Southern Europe, and Northern Africa (Singh, Sharma, Varshney, Sharma, & Singh, 2008).

Figure 1.2. *The two chickpea types; the upper part shows the desi types, and the lower part shows the kabuli types.*

1.9.1.3. Chickpea importance and production

Many attributes suggested to increase chickpea importance as a crop during decades to achieve an effort of sustainable agricultural development by farmers, researchers, and others. (Redden & Berger, 2007), as containing a high nutritive content and is a significant, cheap source of protein in poor nations, making it a part of millions of people's daily diets (Saeed, et al., 2011), in addition to a reliable carbohydrate source which comprised 80 % of dry seed weight (Aggarwal, et al., 2015). Also, seeds contained fiber, calcium, potassium, phosphorous, iron and magnesium (Redden & Berger, 2007), in addition to improve the land fertility (Saeed, et al., 2011) and soil health through symbiotic nitrogen fixation (Thudi, et al., 2016). Additionally, chickpea seed coats and pod covers are used as a fodder source (Aggarwal, et al., 2015).

Many regional names exist for chickpea: *hommos* in the Arab world, *nohut* or *leblebi* in Turkey, *shimbra* in Ethiopia, *garbanzo* in Latin America and *chana* in India (Redden & Berger, 2007). Chickpeas are commonly used in the Mediterranean country's traditional dishes such hummus, falafel, panelle, and farinata (De Giovanni, et al., 2017). Since the 1990s, chickpea seed production has increased, from 7 million tons in 1990 to 11 million tons in 2012, largely due to higher yields, which word widely reached about 0.9 t/ha in 2011 (Aggarwal, et al., 2015). With an annual production of 13.1 million tons,

it was grown over more than 13.2 million hectares worldwide (Thudi, et al., 2016). In 2020, world production of chickpeas was 15 million tons, India produced 73 % of the total production world widely, then Turkey, Myanmar, and Pakistan as secondary producers. The Jordanian chickpea production in 2019 was about 4232 tons with a harvested area as 873 ha, the last record of chickpea production was in 2020 as 3038 tons with a harvested area as 730 ha (http-2).

1.9.1.4. Restrictions and diseases of chickpea

Because there is little genetic diversity in the world's germplasm of chickpea, it lacks the desired agronomic characteristics required for varietal development programs to be used. As a result, the genetic advance for chickpea production is low (Singh, Sharma, Varshney, Sharma, & Singh, 2008). Chickpea has been grown in a wide range of distinct agro-ecological zones, from tropical and subtropical regions where it is grown in the post-monsoonal fall and winter months. In most of its range, it is grown on residual soil moisture following the rainy season, to end of season water shortage and other environmental extremes. The crop matures as temperature increases and moisture decreases in early summer. These environmental challenges, coupled with associated biotic challenges such as the fungal disease Ascochyta blight and Fusarium wilt, which are the essential aspects that affect chickpea yield (Sani, et al., 2018).

For virus diseases, seed born, and non-seed borne viruses are common in chickpeas causes huge economic losses (Makkouk, et al., 2003). Several landraces are still grown in undeveloped areas and valued for their quality. However, this gene pool is gradually being replaced by modern cultivars selected to improve agronomic and economic traits, leaving it extremely vulnerable to the risks of genetic erosion (De Giovanni, et al., 2017). By allowing the transfer of beneficial features from wild types, wild hybridization has played a significant role in the genetic increase of yield in many crops. Utilizing wild species with cultivated varieties gives crop species a larger genetic foundation, perhaps resulting in a supply of resistance genes for various biotic and abiotic stresses (Singh, Sharma, Varshney, Sharma, & Singh, 2008).

Demand for higher-yielding, disease-, insect-, wilt-, and drought-resistant cultivars has been increasing. To meet this demand, chickpea breeders are now turning to their wild relatives for genetic material and has been focused mostly on the closest species to the crop in its gene pools as *C. echinospermum* and *C. reticulatum*, which has resulted in definite improvement. Drought-tolerant traits inherited from the wild progenitors of

chickpeas enable domestic varieties to survive in semi-arid areas without irrigation, minimizing the requirement for irrigation. In some regions of the world, plants have hair-like structures on the stems, leaves, and seed pods that act as a first line of defense against insect pests and save farmers the effort and expense of applying pesticides, in addition to its helping in protect fragile environments (Redden & Berger, 2007).

1.9.1.5. Chickpea diversity

With the geographic spread of the crop, secondary centers of diversity developed, some older than 2000 years in Mediterranean Europe, the Indian subcontinent, and north-east Africa, and some more recently in Mexico and Chile. The primary diversity center is presented in the Fertile Crescent where the crop was domesticated (Redden and Berger, 2007). The five centers of diversity are recognized as:

I. The Mediterranean basin (for kabuli, white seed coat types).
II. Central Asia.
III. West Asia (a secondary center kabuli types).
IV. The Indian subcontinent (for desi coloured seed coat types).
V. Ethiopia (a secondary center for desi types).

South-western Asia is the center of diversity for the nine annual and 35 perennial species that make up the genus *Cicer*. The majority of these species can be found in West Asia and North Africa, which extends from Pakistan in the east to Morocco in the west, and from Turkey in the north to Ethiopia in the south (Singh et al., 2008). Only one of the 44 species is cultivated on a large scale which is the annual *Cicer arietinum* (Redden and Berger, 2007), whereas the majority of the remaining species in the genus are perennials from cooler regions in Central Asia, Anatolia, and the Caucus region (Coyne et al., 2020). Endemic species found in Morocco and the Canary Islands (Redden & Berger, 2007).

The large seeded kabuli domestic types appear to have evolved from the smaller seeded desi types, in association with selection for white seed color and for better food quality. Molecular diversity analyses clearly separate the two groups, which have further subgroups. The distribution of genetic diversity in the kabuli is much narrower than in the desi predominant chickpea type. However, wide genetic diversity occurs in the desi types for flower, pod, seed and vegetative color, variation in seed surface and shape (Redden & Berger, 2007). The nature of *Cicer* and its sexual incompatibility with almost all the wild genotypes in natural interspecific crosses, which is a reason for limited the hybridization process for introgression of economically important traits such as disease

resistance, strength and high seed number/pod which led to reduction in chickpea yield (Rajesh, Sant, Gupta, Muehlbauer, & Ranjekar, 2003).

The National Plant Germplasm System, a collaborative program preserves the genetic diversity of plants, contains over 4000 accessions of chickpea that originated from almost 50 countries. These germplasm resources are made available to scientists world widely to support plant breeding and other research program on chickpea (Redden & Berger, 2007). The ICRISAT has the largest collection as 19,959 accessions of cultivated chickpea and 308 accessions of 18 wild Cicer species from 60 countries. Other major gene banks holding chickpea germplasm include the NBPGR as 16,881 accessions, New Delhi, India; ICARDA as 13,818 accessions, Rabat, Morocco; AGG as 8,655 accessions, Horsham, Victoria; and Western Regional Plant Introduction Station (WRPIS), USDA-Agricultural Research Service (USDA-ARS) as 6,789 accessions, Pullman, and VIR, Russia as 2,091 accessions (Plekhanova et al., 2017). Most accessions within these collections are cultivated material. Furthermore, much of the wild material in international collections has been duplicated, and the true number of independent accessions is shockingly small, with only 18 unique accessions of *C. reticulatum* and 10 of *C. echinospermum* (Coyne, et al., 2020).

In ICARDA, Aleppo, Syria, with a regional delegation for kabuli chickpea, the genebank has 12,070 landrace accessions mainly sourced from Iran (1780), Turkey (970), India (410), Chile (340), Uzbekistan (300), Spain (280), Tunisia (270), Morocco (230), Bulgaria (210), Portugal (170), Russian federation (160), Mexico (160), Jordan (150), USA (120), Bangladesh (110), Tajikistan (110), Azerbaijan (110) and several other regions with less than 100 (Ethiopia, Italy, Palestine, Algeria, Egypt, through to northern Europe, and tropical Africa and South America). Genebanks widely reflect the available sources of genetic diversity in landraces, with a clear superiority in the Indian subcontinent, Iran, the broad stretch of the Middle East and Ethiopia, indicating that important secondary centers of diversification followed the spread of the crop out of the Fertile Crescent (Redden & Berger, 2007).

Chickpea has a little genetic diversity due to obligatory self-pollination and an extensively monotonous genome (Aggarwal et al., 2015). Several bottlenecks occurred during chickpea evolutionary history, which made the genetic basis of cultivated chickpea narrow, and thus a major limitation for chickpea breeding (De Giovanni et al., 2017). The importance of genetic diversity assessment not only for crop improvement but also for

efficient management and conservation of germplasm resource. It is realized that the use of genetically different varieties is an effective strategy to minimize the genetic vulnerability (Pakseresht, Talebi, & Karami, 2013).

1.9.1.6. Chickpea of Jordan

According to Jordan flora that mentioned in Jordan Plant Red List, *Cicer arietinum* is a wild landrace used for cultivated reasons, while *Cicer judaicum* Boiss is a wild relative, extremely rare which are presented in the northern Mediterranean part of the country (Taifour & El-Oqlah, 2015). Chickpea considered a major food legume crop in Jordan and an important part for the daily human diet (Nanish, 2000). Chickpeas are mainly consumed as a breakfast, dinner or as a side dish like Hommus with tahina, Fatteh and Flafel, in addition to many other types of food consumption. In the spring season it is harvested, and consumed row as mature green pods called "Hamleh" roasted with salt and lemon.

Unfortunately, the chickpea production declined in the end of the 1990's due to its low yields and rising the labor costs, that the harvesting is a manual labor which costs the farmers more. So, farmers switched to other crops, such as onion, tobacco, and cereals to increase their profits, as cereal growing is more mechanized. The large part of Jordanian accessions is Kabuli type with 80 %; 68 % of them with semi-spreading growth habit and 92 % with rough testa texture. The evaluated Jordanian chickpea accessions were susceptible to cold, Ascochyta blight and leaf miner. To avoid Ascochyta blight disease and soil moisture, farmers usually delay chickpeas growing from December to spring (February-March), which is the main causes of the low productivity. The crop matures somehow in short growing season, and the plants are subjected to drought with low soil moisture, high evapotranspiration, and heat stress with strong and hot winds. All these reasons affected on chickpeas production (Nanish, 2000).

1.9.2. Lentils

1.9.2.1. Lentil history and distribution

Lentil, *Lens culinaris* Medik. is one of the oldest domesticated, cultivated legume plant, based on archaeological findings; it is the first domestication occurred in Syria and Turkey approximately 8500 BC (Basheer-Salimia, Camilli, Scacchi, Noli, & Awad, 2015). Another archaeological evidence said that Lentil is indigenous to Southwestern Asia and the Mediterranean region back to 7.500 - 6.500 BC. Lentil origin is the Near East and Egypt at the Central and Southern Europe, the Mediterranean basin, Ethiopia,

Afghanistan, India and Pakistan, China and then spread to Latin America (Cokkizgin and Shtaya, 2013). Lentil still traditionally cultivated in the Mediterranean Basin, Asia and has a clear spread in the Americas (Sonnante & Pignone, 2001). It is a winter crop usually grown in fall and harvested in summer; some varieties can also sow in spring (Cokkizgin & Shtaya, 2013).

1.9.2.2. Lentil description

The plant was given its scientific name *Lens culinaris* in 1787 by Medikus a German botanist and physician (Cokkizgin & Shtaya, 2013). It is an annual bushy herb with erect, semi-erect or spreading compact growth habit, varies from 15 to 75 cm in height and has many long ascending branches with soft hairs. The leaves are alternate, with six pairs of oblong-linear, obtuse, mucronate leaflets. The flowers are two to four, small, white, pink, purple, pale purple, or pale blue in color, and are borne in the axils of the leaves, on a slender footstalk nearly equaling the leaves in length, Fig.1.3 shows lentil plant structure. The pods are about 1.5 cm long, slightly inflated, broadly oblong and contain two seeds, which are like the shape of convex lenses about 0.5 cm in diameter (Yadav, McNeil, & Stevenson, 2007).

Figure 1.3. *Illustration of lentil plant parts, Flora von Deutschland, Österreich Und der Schweiz 1885, Gera, Germany.*

There are several cultivated varieties lentils, differing in its size, hairiness, color of the leaves, flowers, and seeds (Yadav, McNeil, & Stevenson, 2007). Taxonomic classification of the genus *Lens* has seven species/subspecies, *L. culinaris* subsp.

culinaris, L. culinaris subsp. *orientalis, L. culinaris* subsp. *tomentosus, L. culinaris* subsp. *odemensis, L. ervoides, Lens lamottei*, and *L. nigricans*. Studies indicated that *L. culinaris* ssp. *orientalis* is the most closely related wild progenitor of *L. culinaris* subsp. *culinaris* (Coyne, et al., 2020). Another author mentioned that "there are a range of wild lentils but *L. culinaris* ssp. *orientalis* is believed to be the progenitor of the cultivated lentil" which can be found in Turkey, Syria, Lebanon, Palestine, Jordan, Iraq, Iran, Afghanistan, Greece, and Uzbekistan (Yadav, McNeil, & Stevenson, 2007).

All *Lens* members are a diploid (2n = 14) annual herbaceous plant, autogamous (self-pollinated) species (Fikiru et al., 2007; Basheer-Salimia et al., 2015). The cultivated *L. culinaris* has two distinguished varieties, based on seed shape and dimension: small, seeded variety (less than 6 mm in diameter) called microsperma and large seeded variety (over 6 mm) called macrosperma (Sonnante & Pignone, 2001; Coyne et al., 2020), macrosperma has 12 varieties and microsperma has 46 varieties (Cokkizgin & Shtaya, 2013). Small-seeded red lentils are South Asian landraces generally early mature types, and the large-seeded are West Asian landraces mostly yellow colored and late mature types (Coyne, et al., 2020).

1.9.2.3. Importance and production of lentil

Lentil is not a major crop, but it is an essential crop in the local agricultural system, grown under rain-fed conditions (Basheer-Salimia, Camilli, Scacchi, Noli, & Awad, 2015). It provides an essential portion of dietary energy supply on a regional basis (Sonnante & Pignone, 2001). Lentil seeds are an important food source for humans, especially in developing countries due to their high-quality protein and fiber content, as well as to their value as straw for animal feed (Basheer-Salimia, Camilli, Scacchi, Noli, & Awad, 2015). Lentils have a good amount of calcium, vitamin A and B, in addition to a good source of iron and phosphorus. It is widely used in a range of dishes and as a side dish, in salads, soups and stews, and assumed to have many uses in traditional medicine. Lentil as an English name has a lot of different common names all over the world as *adas* in Arab Countries, *mercimek* in Turkey, *messer* in Ethopia, *massur* in India, *heramame* in Japanese and in Persian areas as *mangu* or *margu* (Yadav, McNeil, & Stevenson, 2007).

The important lentil growing countries world widely are India, Canada, Turkey, Bangladesh, Iran, China, Nepal, and Syria (Cokkizgin & Shtaya, 2013). The major lentil producing regions are Asia with 58 % and the West Asia-North Africa region with 37 % (Fikiru, Tesfaye, & Bekele, 2007). The prohibitive cost of lentil production in North

Africa and the Middle East has caused a large decrease in its production as in Jordan and Syria (Yadav, McNeil, & Stevenson, 2007). Agronomically lentil is one of the most important grain legumes with a global production of 6.3 million tons in 2016, primarily contributed by Canada as 50.8 %, India as 16.8 %, Turkey as 5.8 % and USA as 4.0 % (Cao, Li, Kapoor, & Banniza, 2019). The global production of lentil in 2020 was 6.5 million tons Canada and India produced 45 % and 18 % respectively of the total world production, in addition to Australia, Turkey, USA, and Nepal. Jordanian lentil production in 2019 was 153 tons and the harvested area was 45 ha. The last record of Jordanian lentil production was in 2020 as 113 tons with the harvested area was as 38 ha only (http-2).

1.9.2.4. Restrictions and diseases of lentil

Abiotic and biotic stresses are the main reasons in reducing the lentil potential yield. Breeding efforts had been focused on the development of varieties with resistance to these stresses. The main breeding strategy has been depended on selection of superior individuals came from crosses between selected breeding cultivars. Due to the extreme selection of certain features of closely related breeding lines, the genetic variability of lentil germplasm has been narrowed. Crop wild relatives are a resource for genetic diversity, assessed by natural selection in a range of environments. It is reasonable to prioritize the wild landraces and their uses for breeding, providing sources of disease resistances; tolerances of drought, heat, frost, and salinity abiotic stresses; and food quality traits (Coyne, et al., 2020).

Wild species are endemic to a wide range of environments and hold many valuable sources of novel variation for yield features including resistance to diseases and abiotic stresses. *L. culinaris* subsp. *orientalis* accessions have been found to contain resistance to drought, cold, vascular wilt, and Ascochyta blight (Yadav, McNeil, & Stevenson, 2007). Wild relatives of lentil offer drought tolerance in *L. nigricans*, *L. odemensis*, and *L. ervoides*, while cold tolerance that can be found in *L. culinaris* subsp. *orientalis*. *L. ervoides* has been identified as a useful source of growth habit, quality seeds and biomass production (Coyne, et al., 2020).

In general, fungal diseases are the most devastative on lentil as wilts, bights, rust, and molds are the most problematic ones. As it is a winter crop, disease risks increase with humid rainy weather. *L. ervoides* exhibited superior resistance to *stemphylium* blight than *L. culinaris*, also resistance had been identified in *L. ervoides* to other diseases such as ascochyta blight and anthracnose (Cao, Li, Kapoor, & Banniza, 2019). Lentil plants

can also be infected by seed born and non-seed borne viruses causing economic losses but the effect on yield losses is not as great as that caused by fungal pathogens (Makkouk, et al., 2003; Yadav, McNeil, & Stevenson, 2007).

1.9.2.5. Lentil diversity

Centers of lentil diversity are characterized by high population density. The Middle East is the primary center of the domestic *Lens culinaris* and its wild progenitor *L. culinaris* subsp. *orientalis* diversity, but taxa are found from Spain to Tajikistan. *L. culinaris* subsp. *orientalis* has two centers of existence; the first is south-eastern Turkey to north-western Syria, and the second is southern Syria to northern Jordan. The southern center overlaps with the center of *L. culinaris* subsp. *odemensis* diversity. *L. culinaris* subsp. *orientalis* accessions from northern Turkey, central Asia and Iran are all remarkably similar to each other. *L. nigricans* diversity region existed in south-western Turkey, while *L. ervoides* diversity region exists along the eastern Mediterranean coast. *L. ervoides* populations from the coastal region of the former Yugoslavia shows a narrow genetic base, as do *L. nigricans* accessions from the same region in addition to those from France and Spain. In general, wild lentil populations are poor competitors to other wild plants, highly grazable by animals and usually form small, separated populations (Yadav, McNeil, & Stevenson, 2007).

All the crop wild relatives presented and overlapped in Turkey and distributed to other geographical regions, as *L. orientalis* distributed throughout the Fertile Crescent (Syria, Lebanon, Jordan, Palestine) and Cyprus, then northeast to Armenia, Azerbaijan, Russia, and the Czech Republic reaching to Iran, Turkmenistan, Uzbekistan, and Tajikistan. *L. odemensis* had limited distribution in Syria and Palestine. *L. tomentosus* found only in Turkey and Syria while *L. ervoides* is found in Syria, Jordan, Palestine, to the west reaching Italy, Croatia, and Montenegro, then to the north Armenia, Azerbaijan, Ukraine, and Russia. *L. nigricans* presented also in Syria, then to the west reaching Greece, Italy, France, Spain, Montenegro, and Croatia, northwards to Ukraine and Crimea, then to the east reaching Bahrin. *L. lamottei* has a limited distribution found in Spain and France (Coyne, et al., 2020).

Genetic variation in available germplasm collections of lentils has been widely used to struggle biotic and abiotic stresses. Cultivated lentils exhibit a lack of variability whereas, they are specific ecotypes in the most important lentil production regions of the world. According to previous studies lentil have seven taxa grouped into four gene pools,

as *L. culinaris* is in the primary gene pool, while *L. orientalis* in the secondary, *L. tomentosus* and *L. lamottei* in the third, *L. odemensis*, *L. ervoides*, and *L. nigricans* were in the fourth gene pools (Coyne et al., 2020). Breeding progress within the cultivated types became limited because of the bottleneck which has reduced genetic variability. Some desirable sources of resistance to drought and fungal diseases have been found in the wild relatives, but it shows a difficulty while crosses because of post-fertilization barriers (Yadav, McNeil, & Stevenson, 2007).

Crossing between cultivated lentil with the wild *L. orientalis* and *L. odemensis* is possible. Although crossing is possible between cultivated lens and the remaining wild species, but results in high possibility of hybrid embryo abortion, sterility, and albino seedlings (Coyne, et al., 2020). High genetic diversity had been found within *L. nigricans*, *L. odemensis* and *L. culinaris* subsp. *orientalis* wild relative to cultivated lentil. But *L. lamottei* and *L. ervoides* were found to have more genetic restrictions (Yadav, McNeil, & Stevenson, 2007), as hybrid embryo breakdown which was rescued allowing to transfer anthracnose resistance gene from *L. ervoides* to *L. culinaris* (Coyne, et al., 2020).

Lentil genotypic characterization have clearly shown different clusters between cultivated and wild germplasms. World widely, the lentil collection was reported as 58,405 accessions held in various national and international seed genebanks with a vast number of duplications. ICARDA genebank conserves a collection of 14,577 lentil accessions including 11,203 landraces, 602 wild accessions, and 2,755 breeding lines. The second diverse collection of lentil germplasms located in NBPGR New Delhi-India with 7,712 lentil accessions, and in the other genebanks such as, the European Cooperative Program for Plant Genetic Resources (ECPGR) holds 4,598 accessions, the AGG presented in Australia about 5,254 accessions, in USDA-ARS 3,187 lentil accessions, and Vavilov Institute in Russia 2,556 accessions (Coyne, et al., 2020).

1.9.2.6. Lentil of Jordan

Three species of lentil found in Jordan, *Lens culinaris* as cultivated landrace, *L. culinaris* subsp. *orientalis* as crop relative and cultivated type and *L. ervoides* as wild landrace (Taifour and El-Oqlah, 2015). Lentil considered as a major food legume crop which consumed by the Jordanian people and an important part for their daily diet. It is consumed as lentil soup or as a main dish named *Mujadara*, which served as a popular dish rich in cheap source of protein. Jordan imports half of its lentil requirements as production declined in the end of the 1990's due to the low yield and rising the labor cost,

that the harvesting done manually which costs the farmers more (Nanish, 2000). In that period the total harvested area was about 3800 ha, while in 2020 the harvested area was declined to 38 ha only (http-2).

Farmers usually delay lentil plantation from December to spring (February-March), which caused to low productivity. The crop matures in short growing season, and the plants are subjected to drought with low soil moisture, high evapotranspiration, and heat stress with the begging of summer hot strong winds. All these reasons affected on lentil production. The Jordanian lentil accessions were evaluated by ICARDA and found that they were among the earliest flowering and maturing accessions, which produced a high amount of seed yields. The Jordanian lentil germplasms were found their tolerance to cold, iron deficiency and Fusarium wilt (Nanish, 2000). Nowadays, NARC conserved in its seed genebank 97 of lentil accession ranged as 75 accessions for *Lens culinaris* and *culinaris* subsp. *orientalis*, and 22 accession for *L. ervoides.*

1.10. Almond

1.10.1. Almond history and distribution

The sweet almond (*Prunus dulcis*, *Prunus amygdalus* or *Prunus communis*), and the bitter almond (*Amygdalus communis*) are a species belonging to the family Rosaceae (Mandalari et al., 2008; La, 2009), belongs to the subgenus *Amygdalus* of the genus *Prunus*, which comprises all stone fruit species. Generally known as nuts, thus occupying a very special place among fruit trees (Fernández i Martí, Font i Forcada, Kamali, Rubio-Cabetas, & Wirthensohn, 2015). The name of this tree came from the Greeks as *Amygdalos,* an almond tree, a word of unknown origin, came from the Semitic language. Taxonomically, almond trees are an Irano- Turanian complex including more than 30 species, but historically, almond trees have played a cultural key and an economic role in Mediterranean human communities. One of the major centers of agriculture origin is the Mediterranean basin; with the first domestication events occurring in the Fertile Crescent about 12000 years before present, followed by agricultural practices spread along the European and African Mediterranean shores (Delplancke, et al., 2013).

Almond originated in Central Asia and over several centuries it spread to the west from its center of origin, towards the Mediterranean Basin. Nowadays it is present grown in the Mediterranean Basin, the Middle East, south-western Asia, USA, and Australia (Fernández i Martí, Font i Forcada, Kamali, Rubio-Cabetas, & Wirthensohn, 2015). Almonds grown world widely in regions that are characterized with a subtropical

Mediterranean climate, with mild wet winters and warm, dry summers (Sorkheh et al., 2007). The seeds are protected by a shell which can be easily transported and stored for a long time. Such potential of long-distance trade has played a central role in the spread and domestication of Almond across the Mediterranean basin (Delplancke, et al., 2016). Most of fruit trees cultivated in the Mediterranean basin (olive, fig, and date palm), almond cultivars have been the long propagated by seeds. It is only since the beginning of the 19th century that vegetative propagation (grafting) was introduced to increase productivity. However, almonds are among the oldest domesticated trees (Delplancke, et al., 2013).

Most *Amygdalus* species occur in southwest Asia while only a few taxa spread into south-east Europe and the central Mediterranean basin. The Levant theory, proposed that the domestication occurred from ancestral wild *P. dulcis* populations, perhaps located in the Fertile Crescent. The other theory debated that domesticated almond trees had a hybrid origin involving several *Amygdalus* species from central/south-western Asia (Delplancke, et al., 2013; 2016). Other reference said that the current cultivated almond is thought to originate from the wild species *Amygdalus communis* that grew in central and south-west Asia admirably adapted to mild, wet winters and dry, hot summers (Harris & Ferguson, 2013).

1.10.2. Almond description

The adult almond tree grows up to 6-10 m height with a trunk diameter about 30-40 cm. In preliminary stages, the almond tree branches are green in color; after exposure to sunlight, they will turn to purplish and then appear gray after some days shown in fig. 1.4. Flowers are white to pale pink color, 3-5 cm in diameters with five petals and are bisexual in nature, whereas leaves are 8-13 cm long with a serrated margin and a 2.5 cm petiole (Laddha, Adki, Gaikwad, & Kulkarni, 2020). The flowers appear early in spring before the leaves. Almond fruits are 3.5-6 cm long, classified botanically as drupes not nuts. The outer covering is a thick, leathery, grey-green coat, with a smooth hairy exterior, called the hull which forms the exocarp and mesocarp together. Inside the hull there is a reticulated, hard shell (woody endocarp) when ripe, inside it the edible seed, which commonly called a nut. One seed presented, but sometimes two seeds can be found. The whole fruit is edible before ripened. After the fruit matures, the hull splits and separates from the shell, and an abscission layer forms between the stem and the fruit so that the fruit can fall from the tree (Doll, 2009).

Figure 1.4. *Illustration of almond parts (A). Almond leaves and fruits in its natural habitat (B).*

The almond seeds are the commercial edible product for human consumption and the dried almond hulls are used for animal feed (Harris & Ferguson, 2013). Almonds are highly heterozygous and highly polymorphic because of its strong self-incompatible system and its dominancy to open pollination (Mir & Patel, 2018), made by insects and mainly by honeybees. The wild types yielded mainly bitter seeds due to the presence of the glycoside amygdalin. Cultivation of gradually sweeter seeds selected from the wild type is thought to have occurred thousands of years ago (Harris & Ferguson, 2013). Almonds widely grown at the Mediterranean area including Jordan, Syria, Turkey and Iraq. In Jordan, the native wild almond (*Amygdalus communis*) with small nuts, hard shell, and bitter seeds grown in the high mountains. Large numbers of almond native genotypes in Jordan show attractive characteristics such as color type, highly adapted to arid and semi-arid regions, drought, and disease resistance, so used as a rootstock for sweet almond and peach, while most of the sweet cultivars were introduced from other countries (La, 2009).

1.10.3. Importance and production of almond

Almond seeds have high nutritive value, high lipid content with concentrated energy sources, which can be used in many food products (La, 2009). They are commonly known as “the king of nuts”, rich in proteins, minerals (iron, manganese, copper, zinc, magnesium, potassium and phosphorus), E and B vitamins, monounsaturated fatty acids,

polyunsaturated fatty acids, and arginine are most widely presented. It contains around 49 % oils, of which 62 % omega-9 fatty acid, 24 % polyunsaturated omega-6 fatty acid, and 6 % saturated fatty acid (Laddha, Adki, Gaikwad, & Kulkarni, 2020). Almonds can be eaten raw (green almond), roasted and, sometimes flavored seeds, with its brown skin. Used in enormous types of food products from cereals to snack foods and pastry products. Various forms of almonds are sold, including blanched, sliced, and chopped as well as pastes, butters and flours and these forms can be incorporated into salty as well as sweet products. In addition to almond's oil and flavonoids used in cosmetics and pharmaceutical products (Harris & Ferguson, 2013).

Almond tree is one of the oldest domesticated nuts trees with the largest commercial production (Sorkheh, et al., 2007). The main outcome of almond urbanization was to increase the seed size, loss of its bitterness and progress types with softer and thinner shells (Delplancke et al., 2016). As mentioned in "FAO, 2008" the annual global almond production exceeds 1.83 million tons, with half of it being produced in the native areas (Delplancke, et al., 2013). California's production estimates 60-80 % of the world production of an over 6000 producing farms in USA with around 30 major commercial varieties. Australia grades the second, followed by Spain and a modest production in other countries on the Mediterranean coast (Greece, Italy, Algeria, Morocco, Syria, Tunisia, and Turkey), Iran and China. The harvesting process begun from July up to November, depending on the variety (Harris & Ferguson, 2013). In 2020, the world production of almond (with shell) was about 4.1 million tons, led by the USA supplying 57 % of the world total production. Other leading producers were Spain, Australia, Iran, Turkey, Morocco and then Italy. In Jordan, the local production of almond (with shell) for 2019 yields was 1409 tons with the harvested area around 363 ha, in 2020 the production increased to 1807 tons in the same size of harvested area (http-2).

1.10.4. Risks and diseases of almond

Almonds are often affected by the spring frost because of its early blooming, because of that several breeding programs have focused on the development of self-compatibility and delayed blooming cultivars (Mir & Patel, 2018). Wild or cultivated types of *Amygdalus communis* in general attacked by many kinds of plant diseases and parasitic weeds which cause severe growth damage, yield failure and in many cases death of the host plants and represent a major threat to fruit and forestry species in various parts of the world and a serious threat to agriculture in Jordan. The major types of parasitic

weeds attack *A. communis* in Jordan are *Cuscuta* sp., *Orobanche* sp., *Viscum cruciatum* and *Osyris alba* (Qasem, 2011). As for virus diseases; prunus necrotic ringspot virus and prune dwarf virus are among the major viral diseases posing a threat to almond production because of their highly quick spread across the field even after good management practices (Mir & Patel, 2018).

Capnodis tenebrionis is one of the most important pests for stone fruits in the Mediterranean area, mainly in drought conditions. Almonds among all stone fruits are the most susceptible to *Capnodis*, due to the biological cycle of the insect and the difficulty of chemical control (Soler, Torrents, & Dicenta, 2013). More than 10 species of *Phytophthora* are known to attack almond trees, causing root rot and cankers on the root crown or trunk. *Phytophthora niederhauserii* was documented killing almond trees in nurseries in Spain. After 2013 *P. niederhauserii* was report in California causing death of almond trees in orchards (Browne, Schmidt, & Brar, 2015). Another study found that an almond field in Adıyaman Province, Turkey was severely infected from root and crown rot, which was the first report of *Phytophthora chlamydospora* infection in the country represents a new host for this pathogen, which within brief time dramatically the field were collapsed and destroyed (Türkölmez, Derviş, Çiftçi, & Ulubaş Serçe, 2016).

1.10.5. Almond diversity

We can say that almonds are the most diverse species among all fruits of its' genus, and this diversity may not only be due to its self-incompatibility and the utilization of open-pollinated seedlings in traditional almond culture, but also because almond is the most southern *Prunus* species that adapted to more diverse growing conditions than other stone fruits, resulting in fitting more diverse microclimates (Fernández i Martí, Font i Forcada, Kamali, Rubio-Cabetas, & Wirthensohn, 2015), such as Mediterranean, steppe, and desert climates characterized by mild, rainy winters and hot, dry summers (Caballero, Trugo, & Finglas, 2003). In addition to those reasons wild to crop gene flow exchange among *Amygdalus* taxa throughout Western and Central Asian might be facilitated because a large part of the group shares a similar diploid chromosome number (2n = 16) which stimulates outcrossing and hybridization (Delplancke, et al., 2012). Although almond have responded well to additional irrigation, fertilizers, other dense cultural methods in addition to insects and disease control that yields can be increased 5-10 folds over the traditional culture practiced for centuries (Caballero, Trugo, & Finglas, 2003).

The Rosaceae family has been considered as outstanding model for testing the gene flow between domesticated and wild species, since hybridization has played a major role in the evolutionary history of the family, resulting in diverse large complex species. Almond, as a part of this family shows many cases of hybridization with other close species, believed its' worth for a gene flow study (Fernández i Martí, Font i Forcada, Kamali, Rubio-Cabetas, & Wirthensohn, 2015). Even though many genetic studies were performed for breeding purposes, little is known about the genetic diversity of almond trees (Delplancke, et al., 2013). Over several centuries the genetic base of almond has been collapsed and special new populations have been established in different growing regions. Locally adapted cultivars are also being lost and converted to modern fields with selected cultivars. Once different breeding programs were started in almond, decreasing in the genetic diversity occurred due to the reduced number of parents used in these programs, leading to the loss of native germplasm throughout the Asian and Mediterranean centers of origin and diversity (Fernández i Martí, Font i Forcada, Kamali, Rubio-Cabetas, & Wirthensohn, 2015).

1.10.6. Almond of Jordan

The native almond species in Jordan mainly have bitter seeds. The Forest Department at Agriculture Ministry of Jordan planted the native almond in many areas for its root stock, seeds, as well as to cover and protect the soil from erosion, furthermore, large numbers of almond native genotypes show attractive characteristics such as color type. In addition, it is used as a rootstock for sweet almond and peach due to its high adaptability to drought and disease resistance (La, 2009). There are differences between the wild type and the sweet type in their external features even before fruit formation; the color of the flowers in the wild/bitter types are pinkish while the sweet types are mostly white in color. The fruits of the wild types are smaller, and the endocarps are harder that the sweet types, in addition to its bitter taste due to the presence of glycoside amygdalin (3-5%) which convert to a toxin substance called prussic acid (Hydrogen cyanide) (Al-Hmoud, Hamasha, & and Al-Kayed, 2003). Wild almond species used by native people for direct consumption, grazing, and oil extraction, in addition to their ecological and socioeconomic importance (La, 2009).

A geographical survey has been conducted three types of wild almond found in Jordan as the following: *Amygdalus communis*, *A. korschinskyi* and *A. Arabica*, most of *A. communis* are presented in the Mediterranean region from north to the south

specifically to Al-Karak region, and some are presented in the Irano-Turanian region which presented around the Mediterranean region. While *A. korschinskyi* presented in the Irano-Turanian region south in Ma'an province, Al-Shobak and Al-tafilah regions. *A. arabica* presented in the desert area (Saharo-Arabian region) in eastern side, particularly in Al-Azraq region (Al-Hmoud, Hamasha, & and Al-Kayed, 2003).

In NARC's seed genebank as sited from its database, more than 100 accession presented as seeds, in addition to *ex-situ* genebank fields presented in different stations in Jordan. 24 cultivars were planted during 1996 in the field genebank of Al-Mushaqar Research Station due to its suitable weather for research purposes (La, 2009). The 24 cultivated accessions was taken from the same field to be tested and determine the genetic diversity of them shown in Fig. 1.6, in addition to 12 wild types collected from different places containing the 3 wild almond types presented in Jordan.

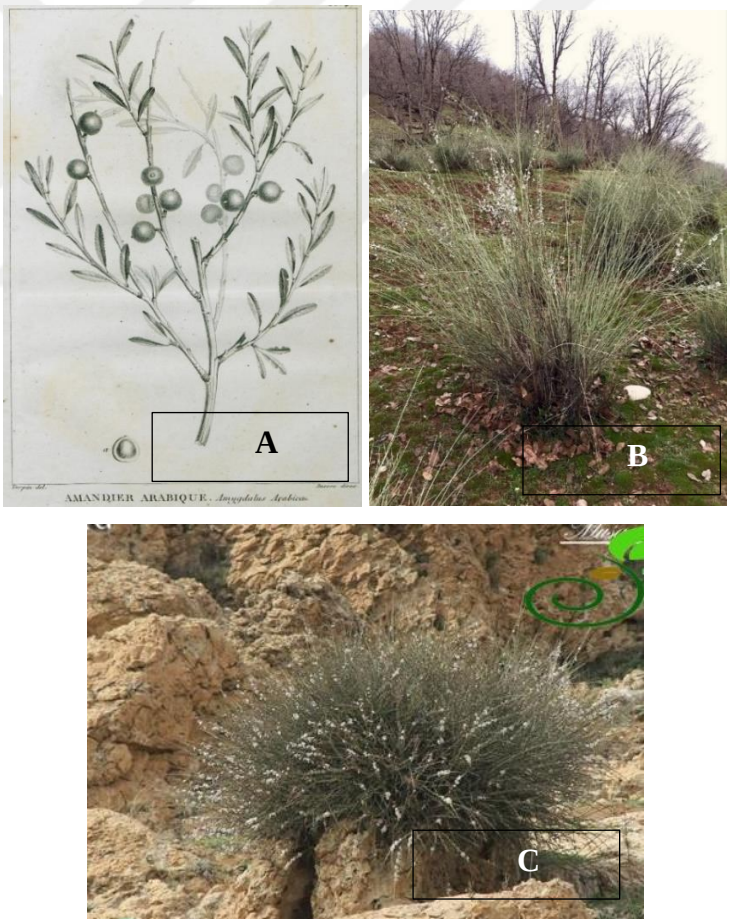


Figure 1.5. *Illustration of A. arabica parts (A). A. arabica growing as shrub in different regions shown in (B+C).*

Figure 1.6. *Almond field genebank of Al-Mushaqar Research Station (NARC).*

Studies proved that the origin of *A. communis* and *A. korschinskyi* is central Asia presented with other types of *Amygdalus*, thus a fusion of their genetic traits occurred as a result of cross-fertilization, which results in new types. So, scientist considered that the wild almond origin of *A. communis* and *A. korschinskyi* presented only in the Levant regions (Syria, Jordan, Lebanon, and Palestine) (Al-Hmoud, Hamasha, & and Al-Kayed, 2003). *Prunus arabica* (*Amygdalus arabica*) is native to the Middle East regions in the desert areas of Iraq, Jordan and Syria (Halász, et al., 2019), in addition it is present in Saudi Arabia and some mountain regions in Turkey and Iran (Yılmaz, Kalkan, & Demirbag, 2020) shown in Fig. 1.5. These wild types of almonds are important for selecting the desirable traits as resistant to drought, salinity, and pests. The bitter almond oil is important medically after removing the toxic cyanide, diluted with other oils using in relieving bone pain and other medical uses, also used in the manufacture of some cosmetics as well (Al-Hmoud, Hamasha, & and Al-Kayed, 2003).

1.11. Modern Aspects

More than a century ago, the concept of genetic resources frameworks breeding, and conservation involved into a genocentric perspective with their own erosion and the danger of losing biodiversity (Aubry, 2019). Using of morphological characterizations for cultivar identification is debatable due to the environmental effects (Gouta, et al., 2010), though; highlight the genomics approaches to genebank management and pre-breeding can facilitate utilization and characterization of PGRs, by providing a vision for the transformation of germplasm collections into bio-digital resource centers (Mascher, et al., 2019). The appearance of plant molecular genetics in the late 1980s made a rapid

increase in the number of studies in which plant germplasm was analyzed by using molecular genetic markers (Henry, 2006). Newly developed technologies as molecular biology-based technology, are greatly participate in the conservation, exploration, and characterization of PGRs, opening new and novel opportunities for their sustainable utilization (Sonnino, 2017).

For effective *in situ* and *ex situ* conservations, molecular markers can be used in biological questions (Kresovich, Williams, McFerson, Routman, & Schaal, 1992). These include:

• Identity: It determines whether an accession or individual is catalogued correctly, is true to type and maintained properly. It can also detect whether genetic change or erosion has occurred in an accession or population over time.

• Similarity: It measures the degree of similarity among individuals in an accession or between accessions within a collection.

• Structure: It also values the partitioning of variation among individuals, accessions, populations and species. Genetic structure is influenced by *in situ* demographic factors such as population size, reproduction and migration.

• Detection: It detects the presence of a particular allele or nucleotide sequence in a taxon, genebank accession, *in situ* population, individual, chromosome or cloned DNA segment.

Genotypic information for all accessions presented in the genebanks will support the germplasm accession and lead the conservation decisions (Mascher, et al., 2019). Genetic techniques and biotechnology tools allowed us to take advantage of the information held in genebanks in a more effective and fast way. Thus, genotyping of the collections, will allow to identify the duplication, varietal limits establishment, estimation of the population variability, identification of haplotypic blocks and other benefits (Díez, et al., 2018). There have been significant improvements in DNA sequencing technologies, which are leading many areas of plant science. The current genomic revolution provides the cost-effective tools that support study of genetic diversity, identify desirable alleles and genes, in addition facilitating their transfer during crop improvement, so reducing the time to hand over new varieties (Wambugu, Ndjiondjop, & Henry, 2018).

Molecular genetic studies have helped issues of the phylogenetic or geographic origins of grouping plant species, that they are significantly increasing for understanding gene bank material (Henry, 2006). A huge majority of recent improvements in biology, medicine, and agriculture were achieved by sharing the accessible sequencing data freely,

which was possible for the massive success of modern genomics and advances of synthetic biology (Aubry, 2019). Future genebanks should conserve products of genetic editing with the associated information, as well as DNA conservation with all the information related to the sequences of the symmetrical genomes (Díez, et al., 2018).

1.12. DNA-Based Molecular Markers

Molecular markers have succeeded in identification among accessions, clarifying synonyms, characterizing mislabeled cultivars, establishment of genetic similarities or geographical origins and giving hints about the process of domestication. However, morphological, and biochemical studies were unable to confirm the relatedness due to their exposure to environmental effects (La, 2009). Molecular markers are selectively neutral, usually located in noncoding regions of DNA in a chromosome, which are unlimited in number and are not affected by environmental factors or the developmental stage of the plant (Govindaraj, Vetriventhan, & Srinivasan, 2015).

Molecular markers can be more effective than previously used identifiers to quantify genetic relationships among germplasms (Henry, 2006). These markers are beneficial more than normal morphological markers as they are stable, also they can increase the speed and accuracy of breeding programs (Törün & Sözen, 2018). Molecular genetic markers can play a large role and can be useful in gathering information on crop taxonomy and evolution, on the geographical and ecological aspects of genetic diversity and patterns of variation. Also, they have been useful in genebank management as in identifying duplicate accessions (Henry, 2006). Molecular markers can be divided into three classes based on their detection method: hybridization based, polymerase chain reaction (PCR) based, and DNA sequence based molecular markers (Govindaraj, Vetriventhan, & Srinivasan, 2015).

1.12.1. Restriction fragment length polymorphism (RFLP)

RFLPs are hybridization-based markers first developed for human-based genetic studies then they were used in plant research in a large-scale comparing genome in the major cereal families such as rye, wheat, maize, sorghum, barley, and rice. It is based on the variations of DNA fragments length that produced by a digestion of genomic DNAs and hybridization to specific markers of two or more individuals of a compared species. The advantages of RFLPs are detecting unlimited number of loci, codominant, strong, and the results are credible transferable across populations. However, the disadvantages are; expensive, consuming time, intensive labor needed, required huge amounts of DNA,

limited polymorphism especially in closely related lines (Govindaraj, Vetriventhan, & Srinivasan, 2015). Most of the early plant genetic maps were based on the use of RFLPs but because it gives only one locus assayed per hybridization experiment, this method is not ideally suited to analyze larger sets of plant germplasm (Henry, 2006).

1.12.2. Randomly amplified polymorphic DNA (RAPD)

PCR-based marker systems are more rapid and require less plant material for DNA extraction. The first PCR-based marker was RAPD which produced by PCR machines using genomic DNA and random primers which create multiple copies of DNA strands. The advantages of RAPD consist in; only small amount of DNA required with the possibility of multiple loci existing from a single primer, in addition of being quick, simple, and in-expensive (Govindaraj, Vetriventhan, & Srinivasan, 2015). In the other hand, RAPD is a dominant marker and non-transferable between laboratories, for these reasons several other types of PCR-based markers became more preferrable (Gouta, et al., 2010).

1.12.3. Amplified fragment length polymorphism (AFLP)

Another type of PCR-based marker which combined both of PCR and RFLP together, by digesting the PCR amplified fragments using specific restriction enzymes that cut DNA at near specific recognition site in nucleotide sequence. AFLPs are extremely reproducible, making it an attractive technique for identifying polymorphisms and for determining linkages by analyzing individuals from a segregating population (Govindaraj, Vetriventhan, & Srinivasan, 2015). AFLPs has created the opportunity for broad genetic characterization of germplasm collections, although emerged as a powerful tool for DNA fingerprinting, molecular characterization, genetic mapping, and extremely high repeatable because of their high polymorphism and multiplicity (Sorkheh, et al., 2007).

1.12.4. Simple sequence repeat (SSR)

Benefiting from the available reference-genome assemblies for almost all major crop species, sequence-based genotyping overcomes the reproducibility issues of earlier marker platforms such as RAPDs or AFLPs (Mascher, et al., 2019). SSR or microsatellite markers are DNA segments containing short sequence repeats and are the preferred technique within crop species due to their plenty, codominance, high polymorphism, and easily used with the PCR conditions (Fernández i Martí, Font i Forcada, Kamali, Rubio-Cabetas, & Wirthensohn, 2015)). For these reasons SSR became the preferred marker for

a wide range of applications in genetics and plant breeding (Gouta, et al., 2010). The different PCR markers detect varying levels of genetic polymorphism; SSRs are the most polymorphic markers, followed by AFLP, which have the advantage of detecting large numbers of loci per gel track, while RAPD is less polymorphic than either and detects fewer loci than AFLP (Henry, 2006).

1.12.5. Inter simple sequence repeat marker (ISSR)

ISSR is based on amplification of genomic segments flanked by inversely oriented and closely spaced SSR loci using microsatellite core unit bearing oligonucleotide primers that could be non-anchored or anchored to 5' or 3' end of the repeats with 1–3 random nucleotides. This technique does not require prior knowledge of DNA sequence, fast, and not expensive (Pakseresht, Talebi, & Karami, 2013; Aggarwal, et al., 2015; Gautam, Gupta, Bhadkariya, Srivastava, & Bhagyawant, 2016). It is another type of DNA sequence-based marker that developed and employed in various genetic studies. ISSRs are dominant markers, but they have several advantages such as high reproducibility, low cost, no previous DNA sequence data needed and require only a little amount of DNA (Törün & Sözen, 2018). ISSR as a technique is helpful in fields of genetic diversity, gene tagging, phylogenetic studies, genome mapping and evolutionary biology in a wide range of crop species (Tahir & Karim, 2011).

1.12.6. Start codon targeted (SCoT) polymorphism

Recently, new alternative marker technique had emerged as target region amplified polymorphism. Connected with the rapid growth of genomics research, there has been a new direction away from random DNA markers towards gene targeted markers, which is the start codon targeted (SCoT) polymorphism. SCoT polymorphisms were based on PCR using single primers that designed on a short, conserved region as all the plant genes surrounding the ATG translation start codon. However, this technique is like RAPD and ISSR, a single primer used as the forward and the reverse primer. SCoT markers are also dominant markers, small amount of DNA needed, simple, easy to work with, informative and inexpensive (Collard & Mackill, 2009).

1.13. Previous Genetic Variation Studies on Chickpea

In Chickpea, techniques such as AFLP, RAPD and ISSR, increasingly used for diagnostic genomic fingerprinting, genetic and qualitative trait loci (QTL) mapping. ISSR markers can be targeted towards specific sequences, which are reported to be abundant in the genome and can overcome the technical difficulties of RFLP and RAPD. The previous

two markers were unable to address the reliable genetic variation within chickpea (Pakseresht, Talebi, & Karami, 2013). ISSR markers were reported to be more compatible than the RAPD markers, as they were highly reporoducible polymorphic DNA markers able to expose various informative loci from a single amplification and had been successfully used to study diversity and phylogenetic relationships for the last decade (Aggarwal, et al., 2015).

SCoT polymorphism marker was used also in chickpea landraces. For a long time, genetic fingerprinting in chickpeas were inhibited by the little genome variability, which can be facilitated by highly polymorphic functional markers as SCoT marker. ISSR and SCoT techniques were more informative than prior biochemical such as isozymes and storage proteins, in addition to other molecular methods as RAPD markers, which used to study variation and genetic relationships in *Cicer* species (Pakseresht, Talebi, & Karami, 2013). The genetic variation detected by ISSR and SCoT markers within and between *Cicer* species were higher than the amount computed from RAPD and AFLP markers (Amirmoradi, Talebi, & Karami, 2012).

Pakseresht, et al. (2013) mentioned in their study that 40 Chickpea landraces were collected from different geographical locations of north-west of Iran, were amplified using ISSR and SCoT markers. 7 ISSR primers were obtained 49 bands, out of them 32 were polymorphic (65%), with an average of 7 bands per primer. 95 bands were utilized from 10 SCoT primers, of which 65 bands were polymorphic (68 %), with an average of 9.5 bands per primers. The average PIC value of ISSR marker was 0.216 and 0.232 for the SCoT marker. Significant differences existed between *Cicer* genotypes, divided them into three distinct clusters. The accessions from the same geographical regions showed more genetic similarities than those from different or isolated places. According to the observed results SCoT marker was more informative than ISSR marker, and both of them were revealed to be better than previous markers for *Cicer* genotypes diversity assessmentation.

Another study was done on 48 Iranian chickpea genotypes were identified using 9 SCoT primers, a total of 145 bands were amplified, of which 133 were polymorphic and had an average of 14.7 band per primer. The average PIC value was 0.45, showed a high level of polymorphism and diversity. The 48 accessions were divided into three main clusters using the similarity matrix (Hajibarat, Saidi, Hajibarat, & Talebi, 2015). Pagar et al. (2017) tested Six chickpea genotypes in their study, from utilized 15 SCoT primers,

only 4 primers were polymorphic, with an average of 8 bands per primer and the polymorphism was about 45%.

Genetic diversity study on cultivated chickpea (*Cicer arietinum*) and its wild progenitor *C. reticulatum* using RAPD and ISSR markers concluded that ISSR analysis was a reliable refer for genetic diversity estimation than of RAPD marker (Gautam, Gupta, Bhadkariya, Srivastava, & Bhagyawant, 2016). 12 types of *C. arietinum* and one of *C. reticulatum* adopted from ICRISAT, India, used in a study using ISSR marker. 10 ISSR primers were used, and a total of 150 bands were amplified, revealed an average of 21.4 bands per primer (Gautam, Gupta, Bhadkariya, Srivastava, & Bhagyawant, 2016).

The genetic diversity of 35 chickpea breeding lines were studied using 14 SCoT primers, which had yielded 135 bands, out of which 100 bands were polymorphic, an average of 7.14 bands per primer were calculated. The average PIC value was 0.36 per primer. Cluster analysis grouped the 35 chickpea breeding lines into three major clusters (Ahmad & Talebi, 2017).

1.14. Previous Genetic Variation Studies on Lentil

During the last decades, different molecular markers, including RAPD and ISSRs, AFLP, SSR and Single nucleotide polymorphisms (SNPs) have been used to estimate genetic variation in lentil (Basheer-Salimia, Camilli, Scacchi, Noli, & Awad, 2015). Genetic diversity in cultivated and wild lentil has been explored using several methods such as Isozyme analysis for the formation of a core collection, RAPD analysis to study population genetic structure and to assess genetic variation in cultivated and wild material, RFLPs that detected a low level of genetic variation in cultivated lentil, whereas AFLPs detected a ten-fold higher polymorphism as compared to RAPDs. In addition to ISSR technique that has been used to investigate genomic origins, assess genetic diversity in germplasm collections, and to identify cultivars (Sonnante & Pignone, 2001).

Research was done on some wild *Lens* types, seeds of 16 populations were collected from different places in Jordan (*L. ervoides* and *L. culinaris* sp. *orientalis*) using RAPD molecular marker. The study showed high polymorphic variability within some collection sites, a total of 65 unique and polymorphic DNA patterns were developed from those wild types based on 7 different primers. Those collected wild populations were conserved in the NARC seed genebank for further studies (Saoub, Haddad, Sadder, & Syouf, 2010). Yadav et al. (2007) said that the wild lentils *L. orientalis* and *L. odemensis* had greater variability for RFLP and were closer to the cultivated lentil *L. culinaris*. However, a

narrow range of diversity could be obtained within the accessions of *L. ervoides* and *L. nigricans.* Another study showed using SNPs that lentil landraces clustered into 3 different groups according to the ecogeographical origin, South Asia (subtropical savannah), Mediterranean, and northern temperate (Coyne, et al., 2020).

In a study 109 lentil germplasms held at ICARDA, originated from different countries contained cultivated and wild accessions were assessed by using the SSR marker. The total number of alleles detected across all microsatellite loci was 182, with a mean of 13 alleles per locus. By the cluster analysis, two major clusters presented and genetically separated the cultivated accessions in one cluster and the wild accessions in another. In the same study, high genetic diversity was observed within the accessions of *L. culinaris*, of which about 80% are from the north and south of Syria, Turkey, Iran, Jordan, Lebanon, Cyprus, and Palestine, considering parts of these countries are a part of the Fertile Crescent, where lentil was first domesticated (Hamwieh, Udupa, Sarker, Jung, & Baum, 2009).

In another study a lentil collection consisting of 467 wild and cultivated accessions that originated from 10 diverse geographical regions was assessed to understand genetic relationships among different lentil species/subspecies, which were identified using high-confidence SNP markers. A weak correlation was observed between clusters and geographical origins, same observations with previous studies, due to the fact that most lentil accessions originated from the Mediterranean regions and then distributed globally. Five distinct clusters were revealed using the STRUCTURE analysis program, separated the cultivated accessions from the wild accessions. Cultivated *L. culinaris*, and wild lentil accessions (*L. ervoides* and *L. nigricans*) were separated in different clusters, due to their large genetic distance observations (Dissanayake, Braich, Cogan, Smith, & Kaur, 2020).

1.15. Previous Genetic Variation Studies on Almond

Developing of molecular markers for almond are particularly useful to study genetic diversity, genome evolution and structure, and for fingerprinting accessions. In the last decade, the molecular marker technique revealed intensive used, and several marker types have been applied to study almond species. Several studies were carried out to characterize almond cultivars and genotypes diversity originated in specific geographical regions (Halász, et al., 2019). For *P. dulcis* and its wild relatives most molecular studies were done using AFLP, RAPD and mostly SSR. In a study done by La (2009) on almond types as; 16 cultivated sweet types, 23 types from different farmers, 4 unknown and 12

wild types using 6 RAPD molecular primers, showed total of 980 RAPD fragments from them 176 bands were polymorphic with 14.2 % polymorphism. High levels of similarity ranged between 0.500 to 0.000, which was the highest was 0.480 between Fkusii and Tuono (both from Italy) cultivated sweet types. The dendrogram tree divided them into 3 clusters, clustering the 16 sweet-cultivated types together, the second cluster with only one wild type and the third cluster contained the rest of the types divided them in smaller groups.

In a study done by Xu et al. (2004) on 40 almond samples, 36 of *P. communis* cultivars and 4 were from other almond species grown in different regions in China using SSR marker, they were clustered in two different groups, group I contained only the Chinese cultivars plus one from unknown origin while group II contained the cultivars of Mediterranean origin. According to the geographic origin, the Mediterranean almond cultivars have a different evolutionary history from the Chinese almond cultivars. As for ISSR molecular marker few studies had been done on stone fruits in general. As an example, a study in China had been done on *Amygdalus mira* using the SSR and ISSR molecular markers (Xing, Tian, Guan, & Meng, 2015).

Genetic diversity of 38 local almond genotypes was studied using 20 RAPD and 15 ISSR primers, the analysis of molecular variance proved a high genetic differentiation within population as 88% for RAPD and 87 % for ISSR. According to Structure analysis, all the genotypes were divided into two groups (Mahood & Hama-Salih, 2020).

Another study was done on three *P. dulcis* types using RAPD and ISSR markers in Egypt (Mohamed, Ghareeb, Ibrahim, & Hafez, 2020). Until today there is no studies found on almond using SCoT markers, but recently another type of stone fruit *Prunus sibirica* in east Asia (Magnolia) was studied using SCoT marker and showed a high genetic variation within populations (Buer, Rula, Wang, Fang, & Bai, 2022).

Due to self-incompatibility a massive and symmetrical gene flow occurs between wild species and cultivated *P. dulcis*, almond could effectively conserve its genetic variability along its dissemination routes from its origin throughout the continents. Drift in isolated geographic regions was shown to stimulate a modest loss of genetic diversity, which resulted in outcomes same to human selected cultivar improvements due to the frequent use of superior almond genotypes. So, the present results indicate almond domestication avoided genetic bottleneck appearance, however the risk still present in many subpopulations (Halász, et al., 2019). A study in Lebanon had been done on the

phenotypic diversity and morphological characterization of *Amygdalus* species, which found high genetic diversity in *Amygdalus communis*, *A. korschinskyi* and *A. orientalis* (La, 2009).

The gene flow between cultivated almond and wild species growing near to tested populations as *P. arabica* and *P. webbii* were shown to be a critical factor for preserving genetic diversity and differentiation among populations. Several previous studies support the bi-directional gene flow among different almond species (Halász et al., 2019). *Prunus arabica* (*Amygdalus arabica*) is native shrub naturally distributed in the Middle East regions Iraq, Syria, Jordan, (Halász, et al., 2019) and Saudi Arabia in addition to some regions of Turkey and Iran (Yılmaz, Kalkan, & Demirbag, 2020). Natural hybridization of *P. dulcis* with *P. arabica* might have probably occurred in these regions. Using SSR molecular marker revealed that *P. dulcis* and *P. arabica* clustered in separate groups but in the same clade (Delplancke, et al., 2016). According to the archeological and ethnographical reported data, wild almonds (*A. arabica* and *A. orientalis*) have been used for a long time in distinct parts of their distribution area. *A. arabica* and its interspecific hybrids were introduced into Morocco by the Carthaginians between the 5th and 4th century BC or by the Arabs during the 6th and 7th centuries (Halász, et al., 2019).

2. METHODOLOGY

2.1. Plant Material

In our study we used plant materials of *Cicer arietinum*, *Lens culinaris*, *Lens ervoides* and *Amygdalus* sp. obtained from the National Agricultural Research Center (NARC), Jordan: local genotypes of chickpea (*Cicer arietinum*) as seeds, local genotypes of lentil (*Lens culinaris*) as seeds, local wild genotypes of lentil (*Lens ervoides*) as seeds (Fig. 2.1), and cultivated and wild genotypes of almond (*Amygdalus* sp.) as leaf samples. Seeds of *C. arietinum* (19 cultivars) (Table 2.1), *L. culinaris* (27 cultivars) and *L. ervoides* (9 cultivars) (Table 2.2) were demanded from NARC seed bank, Al-Baqa'a Station; under the International Treaty on Plant Genetic Resources for Food and Agriculture (SMTA) (Figure 2.1).

Figure 2.1. *Some of the seed samples obtained from NARC seed bank.*

Table 2.1. *Local genotypes of Cicer arietinum (19) used in this study.*

Working code	Serial No.	Taxonomic Name	Province	Location
C1	**2775**	*Cicer arietinum*	Amman	Amman market
C2	**2777**	*Cicer arietinum*	Balqa'	Salt market
C3	**2779**	*Cicer arietinum*	Mafraq	Mafraq market
C4	**2780**	*Cicer arietinum*	Amman	Origin Maur
C5	**2787**	*Cicer arietinum*	Ma'an	Sharah mountain, farmer collection
C6	**2788**	*Cicer arietinum*	Unknown	-
C7	**2791**	*Cicer arietinum*	Jerash	Jerash market
C8	**2793**	*Cicer arietinum*	Ma'an	Shoubak research station
C9	**2794**	*Cicer arietinum*	Karak	Rabba research station
C10	**2795**	*Cicer arietinum*	Zarqa	Zarqa market
C11	**2796**	*Cicer arietinum*	Ma'an	Shoubak
C12	**2798**	*Cicer arietinum*	Madaba	Madaba
C13	**2799**	*Cicer arietinum*	Irbid	Irbid research station
C14	**2801**	*Cicer arietinum*	Unknown	-
C15	**3588**	*Cicer arietinum*	Unknown	-
C16	**3590**	*Cicer arietinum*	Unknown	-
C17	**4361**	*Cicer arietinum*	Ma'an	Shoubak, Al-Johair village
C18	**4537**	*Cicer arietinum*	Ma'an	Shoubak, Al-Juhair area
C19	**4585**	*Cicer arietinum*	Karak	Mu’tah area

Table 2.2. *Local genotypes of Lens culinaris (27) and Lens ervoides (9) used in this study.*

Working code	Serial No.	Taxonomic Name	Province	Location
L1	**3352**	*Lens culinaris*	Irbid	Al-Nuaimeh
L2	**3353**	*Lens culinaris*	Jerash	Balilah
L3	**3360**	*Lens culinaris*	Irbid	Al-Sarih
L4	**3362**	*Lens culinaris*	Jerash	Al-Majar
L5	**3363**	*Lens culinaris*	Jerash	Marsa'a
L6	**3364**	*Lens culinaris*	Ajlun	Anjarah
L7	**3365**	*Lens culinaris*	Amman	Na'our
L8	**3366**	*Lens culinaris*	Madaba	Al-Mansorah
L9	**3370**	*Lens culinaris*	Madaba	New Husban
L10	**3374**	*Lens culinaris*	Madaba	Al-Mamoneyeh
L11	**3376**	*Lens culinaris*	Balqa'	Al-Saru
L12	**3377**	*Lens culinaris*	Balqa'	Al-Yazeedeyeh
L13	**3379**	*Lens culinaris*	Ajlun	Samta
L14	**3380**	*Lens culinaris*	Balqa'	Al-Maisi
L15	**3384**	*Lens culinaris*	Irbid	Al-Mushger
L16	**3388**	*Lens culinaris*	Irbid	Ramtha, Al-Shajarah
L17	**3390**	*Lens culinaris*	Karak	Al-Qaser
L18	**3392**	*Lens culinaris*	Karak	Zeqebah
L19	**3395**	*Lens culinaris*	Karak	Nseib
L20	**3400**	*Lens culinaris*	Irbid	Ramtha, Amrawah
L21	**3401**	*Lens culinaris*	Irbid	Ramtha, Thnebeh
L22	**3404**	*Lens culinaris*	Ajlun	Sakhrah
L23	**3409**	*Lens culinaris*	Karak	Al-Musherfeh
L24	**3411**	*Lens culinaris*	Tafila	Abu-Banna
L25	**4362**	*Lens culinaris*	Ma'an	Shoubak, Al-Johair village
L26	**4538**	*Lens culinaris*	Ma'an	Shoubak, Al-Juhair area
L27	**4339**	*Lens culinaris subsp. orientalis*	Ajlun	Anjarah
L28	**3412**	*Lens ervoides*	Ajlun	Bergish
L29	**3415**	*Lens ervoides*	Ajlun	Al-Mansorah
L30	**3426**	*Lens ervoides*	Balqa'	-
L31	**3428**	*Lens ervoides*	Irbid	-
L32	**3429**	*Lens ervoides*	Irbid	-
L33	**3435**	*Lens ervoides*	Ma'an	Shoubak
L34	**3436**	*Lens ervoides*	Tafila	Dana Reserve
L35	**3437**	*Lens ervoides*	Karak	-
L36	**3438**	*Lens ervoides*	Tafila	Al-Qadeseyeh

Almond leaf samples from 24 cultivars were obtained from NARC, Al-Mushagar Station, Um-Albasatin, Madaba, in addition leaf samples from 12 wild almonds were collected from different places in Jordan (Table 2.3). Fresh leaf materials were placed into plastic bags containing silica gel and dried.

Table 2.3. *The cultivated and wild types of almonds (Amygdalus sp.) used in this study.*

Working Code	Species name	Type	Origin	Place	Location
1	**Ahmah**	Cultivated	Syria	NARC	31.773526,35.801969
2	**Primorski**	Cultivated	Russia	NARC	31.773526,35.801969
3	**Douma 14**	Cultivated	France	NARC	31.773526,35.801969
4	**Chellaston**	Cultivated	Australia	NARC	31.773526,35.801969
5	**Douma 3**	Cultivated	France	NARC	31.773526,35.801969
6	**Fillis**	Cultivated	Greece	NARC	31.773526,35.801969
7	**Ferraduel**	Cultivated	France	NARC	31.773526,35.801969
8	**SF 121**	Cultivated	Spain	NARC	31.773526,35.801969
9	**Shami fark**	Cultivated	Hums (Syria)	NARC	31.773526,35.801969
10	**Princesse**	Cultivated	France	NARC	31.773526,35.801969
11	**Ardshwar 86**	Cultivated	France	NARC	31.773526,35.801969
12	**A-4-4**	Cultivated	Spain	NARC	31.773526,35.801969
13	**Dafadii**	Cultivated	Syria	NARC	31.773526,35.801969
14	**Texas**	Cultivated	USA	NARC	31.773526,35.801969
15	**Fkusii**	Cultivated	Italy	NARC	31.773526,35.801969
16	**A-15-5**	Cultivated	Spain	NARC	31.773526,35.801969
17	**Ne-Plus Ultra**	Cultivated	USA	NARC	31.773526,35.801969
18	**Tuono**	Cultivated	Italy	NARC	31.773526,35.801969
19	**Ai**	Cultivated	France	NARC	31.773526,35.801969
20	**A-13**	Cultivated	Spain	NARC	31.773526,35.801969
21	**Douma 25**	Cultivated	Syria	NARC	31.773526,35.801969
22	**Oja**	Cultivated	Local	NARC	31.773526,35.801969
23	**Kock Tender**	Cultivated	France	NARC	31.773526,35.801969
24	**Drake**	Cultivated	USA	NARC	31.773526,35.801969
25	***A.communis***	Wild	Jordan	Ajlun, Ba'un	32.383306,35.730944
26	***A. communis***	Wild	Jordan	Ajlun, Mahna	32.369650,35.759165
27	***A. communis***	Wild	Jordan	Irbid, Naime	32.414615,35.889711
28	***A. communis***	Wild	Jordan	Irbid, Al-Ibrahimiye	32.431573,35.824319

Table 2.3. (Continued) *The cultivated and wild types of almonds (Amygdalus sp.) used in this study.*

Working Code	Species name	Type	Origin	Place	Location
29	***A. communis***	Wild	Jordan	Irbid, Malka	32.677725,35.745732
30	***A. arabica***	Wild	Jordan, Rewshed	(RBG) Tal Al-Rumman	31.998127,35.829873
31	***A. communis***	Wild	Jordan	Amman, Marj Al-Furs	32.056919,35.938263
32	***A. communis***	Wild	Jordan	Zarqa, Al-Kamshe	32.121565,35.906071
33	***A. communis***	Wild	Jordan	Wadi Al-Azraq, Wadi Shu'aib	32.005738,35.748466
34	***A. communis***	Wild	Jordan	Al-Salt	32.025182,35.716686
35	***A. korschinskyi***	Wild	Jordan	Shobak, Shora'an	30.471845,35.526269
36	***A. korschinskyi***	Wild	Jordan	Dana District	30.675750,35.577191

2.2. Seed Germination

Cartoon teacups were filled 2/3 with peatmoss planting soil and 6 seeds from each lentil genotypes and 4 seeds from each chickpea genotypes were placed on top then covered with a thin layer of soil (Fig. 2.2). After irrigating the soil with tap water, the cups were placed into the growth cabinet (Sanyo MLR-350H) at 16/8 hours (light/dark) photoperiod and 24 ± 2°C. After about 10-14 days, leaf samples were taken from the shoots, labeled, and stored at -20 °C until DNA isolation.

Figure 2.2. *Seed germination steps and process*

2.3. DNA Extraction

Before DNA extraction plant leaf samples were ground into a fine powder with mortar and pestle using liquid nitrogen. All the equipments were wiped with 70 % ethanol before grinding each sample. To get fine powder of plant tissues some samples were ground two to three times. After grinding, the leaf powders were transferred into 2 ml eppendorf by using sterilized spatula and stored at -20 °C.

DNA samples were extracted according to the 2X CTAB (hexadecyltrimethylammonium bromide) protocol (Doyle & Doyle, 1990). The 2X CTAB buffer includes a cation detergent that facilitates the separation of polysaccharides during purification, while additives such as polyvinylpyrrolidone (PVP) can aid in removing polyphenols, to have a pure pellet of DNA as a result. To prepare 50 ml 2X CTAB buffer, following materials presented in the table 2.4. were put into a plastic beaker.

Table 2.4. *Components of 2X CTAB buffer*

Chemicals	**Volume**
NaCl	14 ml (1.4M) or 4.095 g salt
Tris 1M (pH=8)	5 ml
CTAB	1 g
EDTA (0.5M)	2 ml
PVP	0.5 g

Distilled water was added to the final volume of 50 ml and mixed thoroughly, then autoclaved. Finally, 200 µl of β-mercaptoethanol was added to 50 ml 2X CTAB buffer after autoclaving.

DNA extraction was carried out with following steps:

1. 2X CTAB buffer was preheated to 65°C in water bath.
2. 700 µl of 2X CTAB were added to the 500 mg of grounded plant material presented in the 2 ml eppendorf tube, mixed with the pipette to make it homogeneous.
3. The eppendorf tubes were incubated at 65°C for 30 min and vortexing them every 10 min.
4. After the incubation period 700 µl chloroform (the same ratio of the CTAB buffer) was added to each sample and mixed by inverting the tubes about 10 min.
5. The eppendorf tubes were centrifuged at 10000 rpm for 10 min. After centrifugation upper phase of each sample was transferred to a new eppendorf tube.
6. Cold isopropanol (kept at -20°C) was added to each sample as 2/3 of the supernatant volume, then eppendorf tubes were inverted several times to precipitate the DNA. Tubes were transferred and incubated at -20°C for at least 2 hours or overnight.
7. The samples were centrifuged at 10000 rpm for 10 min to obtain DNA pellet, the supernatant was poured out carefully without losing the pellet.
8. The DNA pellet was washed twice with 300µl 70 % ethanol, then centrifuged at 10000 for 5 min.
9. The pellets were dried in a sterile cabinet by leaving the eppendorf tubes open. After that 200 µl of DNAs/RNAs free water was added onto each pellet and dissolved DNA either stored at +4°C for a short time or at -20°C for a longer storage.

2.4. Measuring the DNA Quality and Quantity

For measuring the quality of DNA, 4 µl of isolated DNA was taken for each sample and run in 0.8 (Collard & Mackill, 2009)% agarose gel with a 1kb DNA ladder for 30-40 min at 90V, checking whether the quality of the DNAs was suitable to continue the procedure by observing a single bright band in the gel. Then visualized under the UV light and digitally photographed with gel documentation system (Uvitec, Cambridge, UK). DNA isolation was repeated for the samples that form very faint band or smear appearance on the agarose gel.

Measuring the DNA purity and quantification Nanodrop Spectrophotometer was used at 260 and 280 nm wavelengths by reading 2 µl of each sample separately with

DNAs/RNAs free water (used for dissolving DNA pellets before) as a blank. According to the measurement results, DNA samples were diluted with DNAs/RNAs free water as 5 and 10 ng/µl to be used in PCR analyses.

2.5. ISSR-PCR Analysis

ISSR primers designed by the University of British Columbia Biotechnology Laboratories were used for PCR analysis (Table 2.5).

Table 2.5. *The sequences of ISSR primers used in the study.*

Primer code	Sequence (5′-3′)	Primer code	Sequence (5′-3′)
807	AGA GAG AGA GAG AGA GT	826	ACA CAC ACA CAC ACA CC
808	AGA GAG AGA GAG AGA GC	829	TGT GTG TGT GTG TGT GC
809	AGA GAG AGA GAG AGA GG	834	AGA GAG AGA GAG AGA GYT
810	GAG AGA GAG AGA GAG AT	842	GAG AGA GAG AGA GAG AYG
811	GAG AGA GAG AGA GAG AC	845	CTC TCT CTC TCT CTC TRG
812	GAG AGA GAG AGA GAG AA	847	CAC ACA CAC ACA CAC ARC
813	CTC TCT CTC TCT CTC TT	851	GTG TGT GTG TGT GTG TYG
814	CTC TCT CTC TCT CTC TA	853	TCT CTC TCT CTC TCT CRT
815	CTC TCT CTC TCT CTC TG	855	ACA CAC ACA CAC ACA CYT
817	CAC ACA CAC ACA CAC AA	859	TGT GTG TGT GTG TGT GRC
818	CAC ACA CAC ACA CAC AG	861	ACC ACC ACC ACC ACC ACC
819	GTG TGT GTG TGT GTG TA	862	AGC AGC AGC AGC AGC AGC
823	TCT CTC TCT CTC TCT CC	866	CTC CTC CTC CTC CTC CTC
824	TCT CTC TCT CTC TCT CG	870	TGC TGC TGC TGC TGC TGC

Three random DNA samples was selected from each plant type for testing ISSR primers, and the aim here was to determine the ISSR primers that create a clear and reproducible band profile at the end of PCR reactions. For optimization template DNA, Mg^{+2}, primer concentration and primer annealing temperatures were adjusted to which the primers work best. Tables 2.6-2.9 show the used ISSR-PCR reaction and cycling conditions for chickpea, lentil, and almond samples.

Table 2.6. *ISSR-PCR mix for chickpea samples.*

PCR Components	Volume
dH_2O	6.9 µl
10X Taq buffer (Fermentas)	1.5 µl
25 mM $MgCl_2$ (Fermentas)	1.2 µl
2.5 mM dNTP (Fermentas)	1.2 µl
2.5 µM Primer	1 µl
5 ng/ µl Template DNA	3 µl
1U Taq polymerase (Fermentas)	0.15 µl
Total	15 µl

Table 2.7. *ISSR-PCR mix for lentil samples.*

PCR Components	Volume
dH_2O	7.6
10X Taq buffer (Fermentas)	1.5 µl
25 mM $MgCl_2$ (Fermentas)	1.5 µl
2.5 mM dNTP (Fermentas)	1.2 µl
2.5 µM Primer	1 µl
10 ng/ µl Template DNA	2 µl
1U Taq polymerase (Fermentas)	0.15 µl
Total	15 µl

Table 2.8. *ISSR-PCR mix for almond samples.*

PCR Components	Volume
dH_2O	10.3 µl
10X Taq buffer (Fermentas)	2.5 µl
25 mM $MgCl_2$ (Fermentas)	2 µl
2.5 mM dNTP (Fermentas)	1.5 µl
2.5 µM Primer	1.5 µl
10 ng/ µl Template DNA	2 µl
1U Taq polymerase (Fermentas)	0.2 µl
Total	20 µl

Table 2.9. *ISSR-PCR cycling conditions*

	Temperature (°C)	Duration	Number of cycle
Predenaturation	94	4 min	1
Denaturation	94	45 sec	45
Annealing	50-55	1:30 min	
Elongation	72	2 min	
Final elongation	72	5 min	1

PCR reactions were prepared with the amounts indicated in Tables 2.6, 2.7 and 2.8 in a volume of 15 or 20 µl. A negative control (without DNA) was included in each PCR reaction to make sure there is no DNA contamination. PCR reactions were performed in Applied Biosystems Veriti gradient thermal cycler. PCR cycles started with a 4 min predenaturation at 94°C, then 45 cycles of 45 sec at 94 °C, 90 sec between 50-55 °C and 2 min at 72 °C. At the end of those cycles there was a final extension for 5 min at 72 °C. PCR products were stored at +4°C until run on agarose gel.

2.6. SCoT-PCR Analysis

SCoT primer sets (Collard & Mackill, 2009) used in this study were synthesized by Oligomer Biotechnology Company, Ankara-Turkey (Table 2.10).

Table 2.10. *The sequences of SCoT primers used in the study.*

Primer Code	Sequence (5′-3′)	Primer Code	Sequence (5′-3′)
SCoT-1	CAACA**ATG**GCTACCACCA	**SCoT-19**	ACC**ATG**GCTACCACCGGC
SCoT-2	CAACA**ATG**GCTACCACCC	**SCoT-21**	ACGAC**ATG**GCGACCCACA
SCoT-3	CAACA**ATG**GCTACCACCG	**SCoT-20**	ACC**ATG**GCTACCACCGCG
SCoT-4	CAACA**ATG**GCTACCACCT	**SCoT-24**	CACC**ATG**GCTACCACCAT
SCoT-6	CAACA**ATG**GCTACCACGC	**SCoT-25**	ACC**ATG**GCTACCACCGGG
SCoT-9	CAACA**ATG**GCTACCAGCA	**SCoT-26**	ACC**ATG**GCTACCACCGTC
SCoT-10	CAACA**ATG**GCTACCAGCC	**SCoT-28**	CC**ATG**GCTACCACCGCCA
SCoT-12	ACGAC**ATG**GCGACCAACG	**SCoT-32**	CC**ATG**GCTACCACCGCAC
SCoT-15	ACGAC**ATG**GCGACCGCGA	**SCoT-33**	CC**ATG**GCTACCACCGCAG
SCoT-16	ACC**ATG**GCTACCACCGAC	**SCoT-34**	ACC**ATG**GCTACCACCGCA
SCoT-17	ACC**ATG**GCTACCACCGAG	**SCoT-36**	GCAACA**ATG**GCTACCACC
SCoT-18	ACC**ATG**GCTACCACCGCC		

Three random DNA samples was selected for testing SCoT primers for each plant type to determine primers that create a clear band profile. For PCR optimization different concentrations of primer, template DNA, and Mg^{+2} was tested, and also gradient PCR were undertaken to determine the best primer annealing temperature to obtain brighter bands. Tables 2.11-2.13 show the used SCoT-PCR reaction and cycling conditions for chickpea, lentil, and almond samples.

Table 2.11. *SCoT-PCR mix for chickpea and lentil samples*

PCR Components	Volume
dH_2O	6.5 µl
10X Taq buffer KCL (Fermentas)	2.5 µl
25 mM $MgCl_2$ (Fermentas)	2 µl
2.5 mM dNTP (Fermentas)	2 µl
2.5 µM Primer	5 µl
10 ng/ µl Template DNA	5 µl
1U Taq polymerase (Fermentas)	0.2 µl
Total	25 µl

Table 2.12. *SCoT-PCR mix for almond samples*

PCR Components	Volume
dH_2O	8.5-7.5 µl
10X Taq buffer KCL (Fermentas)	2.5 µl
25 mM $MgCl_2$ (Fermentas)	2 µl
2.5 mM dNTP (Fermentas)	2 µl
2.5 µM Primer	5 µl
10 ng/ µl Template DNA	3-4 µl
1U Taq polymerase (Fermentas)	0.2 µl
Total	25 µl

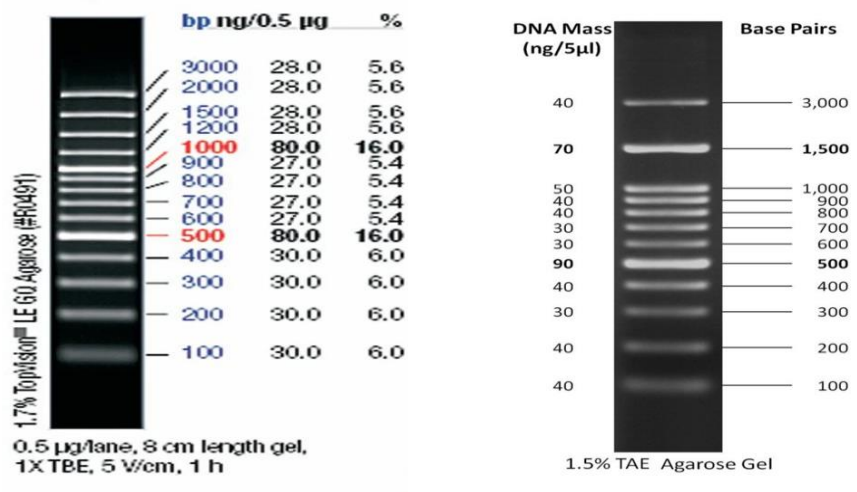
Table 2.13. *SCoT-PCR cycling conditions*

	Temperature (°C)	Duration	Number of cycle
Predenaturation	94	3 min	1
Denaturation	94	1 min	40
Annealing	50-61	1 min	
Elongation	72	2 min	
Final elongation	72	5 min	1

PCR reactions were prepared with the amounts indicated in Tables 2.11 and 2.12 in a volume of 25 µl. A negative control (without DNA) was included in each PCR reaction to make sure there is no DNA contamination. PCR reactions were performed in Applied Biosystems Veriti gradient thermal cycler. PCR cycles started with a 3 min pre-denaturation at 94°C then 40 cycles of 1 min at 94 °C, 1 min at 50-61 °C and 2 min at 72 °C. At the end of those cycles there was a final extension for 5 min at 72 °C. PCR products were stored at +4°C until run on agarose gel.

2.7. Agarose Gel Electrophoresis

The ISSR and SCoT-PCR products were electrophoresed in 1.8 % agarose gel containing 5µl Red Safe (Intron Bio). Firstly, 8 µl of each PCR product was mixed with 2 µl of 6 X Loading Dye (Thermo) and then loaded into the wells of the agarose gel leaving the first and the last wells for loading the 100 bp plus DNA ladder (5 µl) (either Thermo GeneRuler 100 bp plus or ABM Opti-DNA Ladder 100 bp plus, Fig. 2.3). The gel was run for 1 h and 30 min at 85V in the gel tank (Thermo, Midicell Primo) after that it was visualized and photographed with UV documentation system (Uvitec, Cambridge, UK).



(A) **(B)**

Figure 2.3. *100 bp DNA Ladders used in this study. (A) Thermo GeneRuler Marker 100 bp plus (B)ABM Opti-DNA markers 100bp plus.*

2.8. Band Count and Data Analysis

The gel photographs of ISSR and SCoT PCR analyses were analyzed by using TOTALLAB CLIQS (1D gels) software program. For each primer, the number of bands and their sizes were determined by comparing the band sizes of the 100 bp plus DNA ladder. In addition, a binary matrix was prepared based on the results obtained, where the presence of the band was designated as (1) and its absence as (0) for each sample. The "polymorphism information content" (PIC) of each ISSR and SCoT primers was calculated with the following formula:

PIC = $2f(1-f)$ (De Riek, Calsyn, Everaert, Van Bockstaele, & De Loose, 2001)

In this formula, f is the frequency of alleles. This calculation was used to find PIC value of each band amplified with a specific primer and then average value was calculated.

The genetic similarity values between chickpea, lentil and almond genotypes were calculated by Jaccard coefficient using the DendroUPGMA program. Dendrograms for both ISSR and SCOT markers were constructed using the MEGA software version 11.

Population structure analysis was performed by the STRUCTURE software program v. 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). The software assumed a model of admixture degree for each individual plant and the populations (K) that contribute to the genotype of each individual, which characterized by a set of allele frequencies at each marker locus. The program was run with a burn-in period of 5000 repetition followed by

50000 MCMC burn-in repetition, while K value was ranged from 1 to 10 and the average results of iteration was 10 runs of each K value. STRUCTURE HARVESTER (Earl & VonHoldt, 2012) was used to calculate the K values, delta K graph and to extract the suitable bar plot graph for each plant variety using the binary matrix of ISSR and SCoT markers.

3. RESULTS

3.1. Seeds Germination

Not all seeds, especially chickpea seeds germinated at the same time, some seeds had been rotted and therefore germination process was repeated in some accessions. Although the germination process was repeated 3 times with the C19 coded variety of chickpea, and the seeds rotted each time. Seeds of *L. ervoides* in general did not germinate as fast as seeds of *L. culinaris*. To trigger germination, the planted cups were covered with aluminum foil and kept in the refrigerator at +4°C for another week to break the dormancy. A week later some of them started to germinate, they were replaced back to the incubator to complete their germination. Among lentil accessions seeds of L29 did not germinate after 3 trials. The seeds of C19 and L29 were requested again from NARC, but these seeds also did not germinate after two attempts and these accessions were excluded from our study.

3.2. Measuring the DNA Quality and Quantification

3.2.1. DNA quality measurements

The quality of the DNA was checked by taking 4 µl of isolated DNA for each sample and run in 0.8 % agarose gel with a 1kb DNA ladder for 30-40 min at 90V, visualized under the UV light and digitally photographed with gel documentation system. DNA extraction was repeated for the samples that form very faint band or smear appearance on the agarose gel. Figures 3.1, 3.2 and 3.3 shows the isolated DNA samples of chickpea, lentil, and almond accessions, respectively.

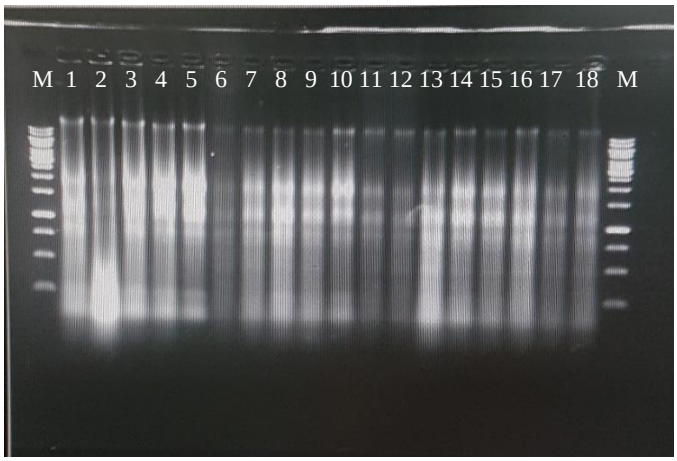


Figure 3.1. *Agarose gel results of DNA quality of 18 chickpea accessions (1-18), M:1kb DNA marker.*

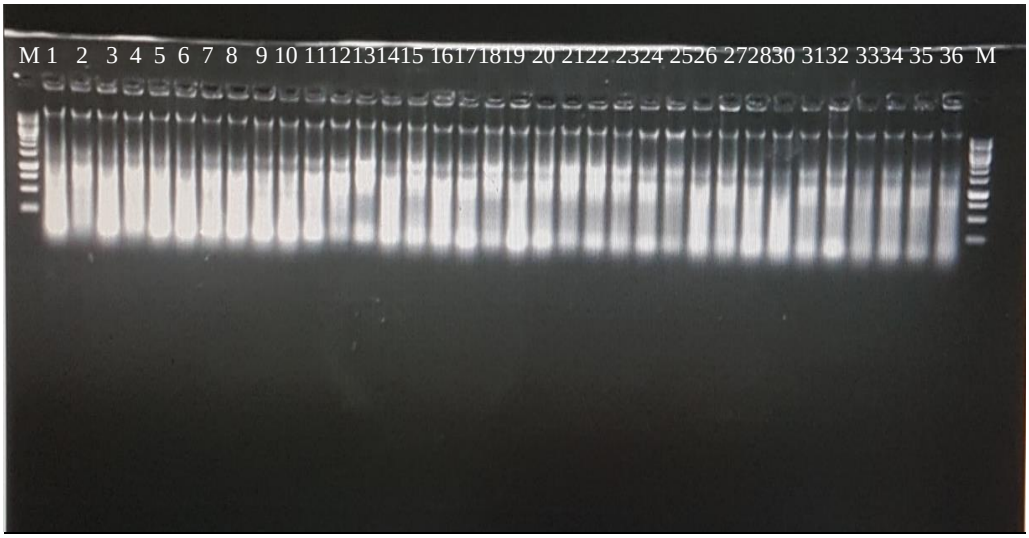


Figure 3.2. *Agarose gel results of DNA quality of 35 lentil accessions (1-35), M:1kb DNA marker.*

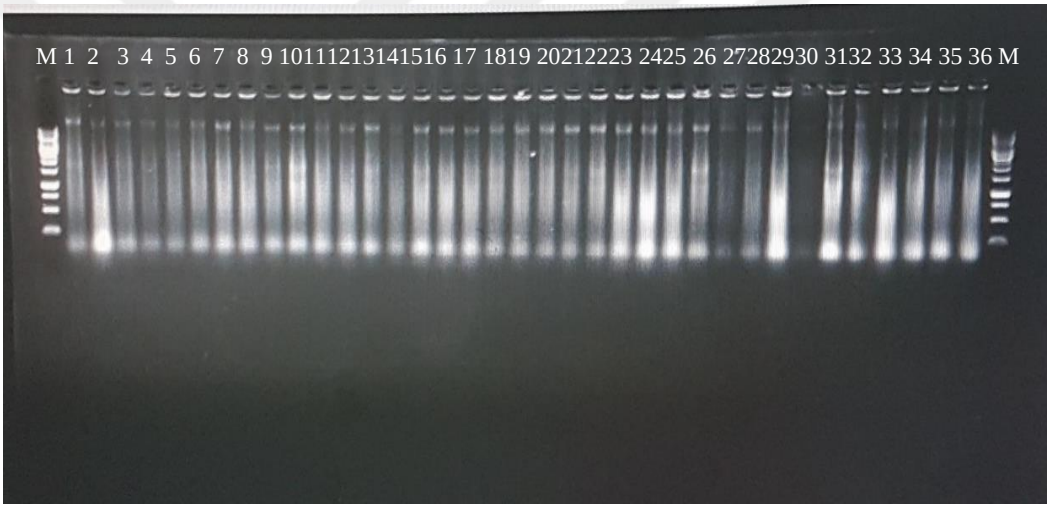


Figure 3.3. *Agarose gel results of DNA quality of almond accessions (1-36), M:1kb DNA marker.*

3.2.2. DNA quantification measurements

In order to measure the DNA purity and quantity of samples, Nanodrop Spectrophotometer was used at 260-280 wavelength and according to the measurement results, DNA samples were diluted with DNAs/RNAs free water in 5 or 10 ng/µl to be used in PCR analyses. Tables 3.1, 3.2 and 3.3 provide information about DNA purity and quantity values of chickpea, lentil, and almond accessions, respectively.

Table 3.1. *DNA purity and quantity values of 18 chickpea accessions.*

Chickpea accessions	Purity 260/280	Quantity ng/µl	Chickpea accessions	Purity 260/280	Quantity ng/µl
C1	2.08	507	**C10**	2.15	1249
C2	2.14	1945	**C11**	2.06	508
C3	2.17	1452	**C12**	2.05	523
C4	1.06	5283	**C13**	2.15	1770
C5	2.1	483	**C14**	2.14	1329
C6	2.14	907	**C15**	2.15	1590
C7	2.14	1071	**C16**	2.15	1438
C8	2.15	1644	**C17**	2.12	786
C9	2.15	1184	**C18**	2.13	665

Table 3.2. *DNA purity and quantity values of 35 lentil accessions.*

Lentil accessions	Purity 260/280	Quantity ng/µl	Lentil accessions	Purity 260/280	Quantity ng/µl
L1	2.12	1188	**L19**	2.09	637
L2	2.02	423	**L20**	2.11	573
L3	2.1	1032	**L21**	2.11	466
L4	2.02	583	**L22**	2.09	520
L5	2.13	991	**L23**	2.12	373
L6	2.08	662	**L24**	2.05	433
L7	2.02	498	**L25**	2.08	348
L8	2.01	546	**L26**	2.07	494
L9	2.05	504	**L27**	2.09	712
L10	2.05	526	**L28**	2.05	172
L11	2.03	487	**L30**	2.01	151
L12	2.03	446	**L31**	2.06	324
L13	1.78	925	**L32**	2.07	562
L14	2.12	891	**L33**	2.07	273
L15	2.15	806	**L34**	2.04	399
L16	2.03	505	**L35**	2.08	370
L17	2.04	527	**L36**	2.07	308
L18	2.11	483			

Table 3.3. *DNA purity and quantity values of 36 almond accessions.*

Almond accessions	Purity 260/280	Quantity ng/µl	Almond accessions	Purity 260/280	Quantity ng/µl
1	2.17	77	**19**	2	61
2	1.97	108	**20**	2.11	68
3	2.17	60	**21**	2	44
4	2.03	38.3	**22**	1.82	65
5	2.1	44.3	**23**	2.15	86
6	2	64	**24**	2.1	133
7	2.02	79	**25**	1.85	90
8	2.05	76	**26**	2.16	62
9	1.99	51	**27**	1.8	23
10	2.2	72	**28**	2.04	42
11	1.86	98	**29**	2.03	104
12	1.89	93	**30**	1.8	35
13	1.68	66	**31**	2.2	150
14	1.96	42	**32**	2.2	82
15	2.04	71	**33**	2.1	69
16	1.96	81	**34**	2.1	104
17	1.82	78	**35**	2.12	120
18	2.01	52	**36**	2.3	62

3.3. ISSR Primer Optimization

In total 26 ISSR primers (Table 3.4) were evaluated for chickpea accessions and 13 ISSR primers were chosen due to amplifying clear and reproducible band profiles.

Table 3.4. *Tested 26 ISSR primers for chickpea DNA accessions.*

	Primer code	Sequences (5'- 3')	Temp. ˚C	Results
1	**ISSR 807**	AGA GAG AGA GAG AGA GT	52	**Full set**
2	ISSR 808	AGA GAG AGA GAG AGA GC	53	Incomplete set
3	ISSR 809	AGA GAG AGA GAG AGA GG	53	Just one band
4	**ISSR 810**	GAG AGA GAG AGA GAG AT	50	**Full set**
5	**ISSR 811**	GAG AGA GAG AGA GAG AC	50	**Full set**
6	**ISSR 812**	GAG AGA GAG AGA GAG AA	50	**Full set**
7	**ISSR 813**	CTC TCT CTC TCT CTC TT	50	**Full set**
8	ISSR 814	CTC TCT CTC TCT CTC TA	50	Did not work
9	ISSR 815	CTC TCT CTC TCT CTC TG	53	Incomplete set
10	**ISSR 817**	CAC ACA CAC ACA CAC AA	50	**Full set**
11	**ISSR 818**	CAC ACA CAC ACA CAC AG	53	**Full set**
12	**ISSR 819**	GTG TGT GTG TGT GTG TA	50	**Full set**
13	ISSR 823	TCT CTC TCT CTC TCT CC	53	Did not work

Table 3.5. (continued) *Tested 26 ISSR primers for chickpea DNA accessions.*

	Primer code	Sequences (5'- 3')	Temp. ˚C	Results
14	ISSR 824	TCT CTC TCT CTC TCT CG	53	Did not work
15	ISSR 826	ACA CAC ACA CAC ACA CC	53	Incomplete set
16	**ISSR 829**	TGT GTG TGT GTG TGT GC	53	**Full set**
17	**ISSR 834**	AGA GAG AGA GAG AGA GYT	53	**Full set**
18	ISSR 842	GAG AGA GAG AGA GAG AYG	55	Just one band
19	ISSR 845	CTC TCT CTC TCT CTC TRG	55	Did not work
20	**ISSR 847**	CAC ACA CAC ACA CAC ARC	55	**Full set**
21	ISSR 851	GTG TGT GTG TGT GTG TYG	55	One light band
22	**ISSR 853**	TCT CTC TCT CTC TCT CRT	53	**Full set**
23	**ISSR 855**	ACA CAC ACA CAC ACA CYT	53	**Full set**
24	ISSR 859	TGT GTG TGT GTG TGT GRC	55	Incomplete set
25	ISSR 861	ACC ACC ACC ACC ACC ACC	61	one light band
26	ISSR 862	AGC AGC AGC AGC AGC AGC	61	Did not work

On the other hand, 23 ISSR primers were screened for lentil and almond accessions (Tables 3.5 and 3.6) and 13 ISSR primers were chosen for further analyses.

Table 3.6. *Tested 23 ISSR primers for lentil DNA accessions.*

	Primer code	Sequences (5'- 3')	Temp. ˚C	Results
1	**ISSR 807**	AGA GAG AGA GAG AGA GT	50	**Full Set**
2	**ISSR 808**	AGA GAG AGA GAG AGA GC	53	**Full Set**
3	**ISSR 809**	AGA GAG AGA GAG AGA GG	53	**Full Set**
4	ISSR 810	GAG AGA GAG AGA GAG AT	50	Did not work
5	**ISSR 812**	GAG AGA GAG AGA GAG AA	50	**Full Set**
6	**ISSR 813**	CTC TCT CTC TCT CTC TT	50	**Full Set**
7	**ISSR 814**	CTC TCT CTC TCT CTC TA	50	**Full Set**
8	ISSR 815	CTC TCT CTC TCT CTC TG	53	Light Bands
9	**ISSR 817**	CAC ACA CAC ACA CAC AA	50	**Full Set**
10	ISSR 818	CAC ACA CAC ACA CAC AG	53	Light Bands
11	**ISSR 819**	GTG TGT GTG TGT GTG TA	50	**Full Set**
12	ISSR 823	TCT CTC TCT CTC TCT CC	53	Did not work
13	**ISSR 824**	TCT CTC TCT CTC TCT CG	53	**Full Set**
14	ISSR 826	ACA CAC ACA CAC ACA CC	53	Light Bands
15	ISSR 829	TGT GTG TGT GTG TGT GC	53	Light Bands
16	**ISSR 834**	AGA GAG AGA GAG AGA GYT	53	**Full Set**
17	ISSR 842	GAG AGA GAG AGA GAG AYG	55	Light-Few Bands
18	ISSR 845	CTC TCT CTC TCT CTC TRG	55	Did not work
19	**ISSR 847**	CAC ACA CAC ACA CAC ARC	55	**Full Set**
20	ISSR 851	GTG TGT GTG TGT GTG TYG	55	Light Bands
21	**ISSR 853**	TCT CTC TCT CTC TCT CRT	53	**Full Set**
22	**ISSR 855**	ACA CAC ACA CAC ACA CYT	53	**Full Set**
23	ISSR 859	TGT GTG TGT GTG TGT GRC	55	Light Bands

Table 3.7. *Tested 23 ISSR primers for almond DNA accessions.*

	Primer code	Sequences (5'- 3')	Temp. ˚C	Results
1	**ISSR 807**	AGA GAG AGA GAG AGA GT	52	**Full Set**
2	**ISSR 808**	AGA GAG AGA GAG AGA GC	53	**Full Set**
3	**ISSR 809**	AGA GAG AGA GAG AGA GG	53	**Full Set**
4	**ISSR 810**	GAG AGA GAG AGA GAG AT	50	**Full Set**
5	ISSR 812	GAG AGA GAG AGA GAG AA	50	One Band
6	ISSR 813	CTC TCT CTC TCT CTC TT	50	One Band
7	**ISSR 814**	CTC TCT CTC TCT CTC TA	50	**Full Set**
8	**ISSR 815**	CTC TCT CTC TCT CTC TG	53	**Full Set**
9	**ISSR 817**	CAC ACA CAC ACA CAC AA	50	**Full Set**
10	ISSR 818	CAC ACA CAC ACA CAC AG	53	Light Bands
11	ISSR 819	GTG TGT GTG TGT GTG TA	50	Light Bands
12	ISSR 823	TCT CTC TCT CTC TCT CC	53	One Light Band
13	ISSR 824	TCT CTC TCT CTC TCT CG	53	One Band
14	ISSR 826	ACA CAC ACA CAC ACA CC	53	Light Bands
15	**ISSR 829**	TGT GTG TGT GTG TGT GC	53	**Full Set**
16	**ISSR 834**	AGA GAG AGA GAG AGA GYT	53	**Full Set**
17	**ISSR 842**	GAG AGA GAG AGA GAG AYG	55	**Full Set**
18	ISSR 845	CTC TCT CTC TCT CTC TRG	55	Light Bands
19	**ISSR 847**	CAC ACA CAC ACA CAC ARC	55	**Full Set**
20	**ISSR 851**	GTG TGT GTG TGT GTG TYG	55	**Full Set**
21	ISSR 853	TCT CTC TCT CTC TCT CRT	53	Light Bands
22	ISSR 855	ACA CAC ACA CAC ACA CYT	53	Light Bands
23	**ISSR 859**	TGT GTG TGT GTG TGT GRC	55	**Full Set**

3.4. Results of ISSR-PCR Analysis

3.4.1. ISSR-PCR analysis of chickpea accessions

Agarose gels containing amplified products of 13 ISSR primers from 18 chickpea accessions were visualized under the UV light and digitally photographed with gel documentation system (Figures 3.4-3.16).

ISSR 807: A total of 10 bands were amplified with primer ISSR 807 (Figure 3.4). The largest band size was 1790 bp and the smallest band was 566 bp, the polymorphism percentage of the primer was 70 % and the Polymorphism Information Content (PIC) value was 0.17.

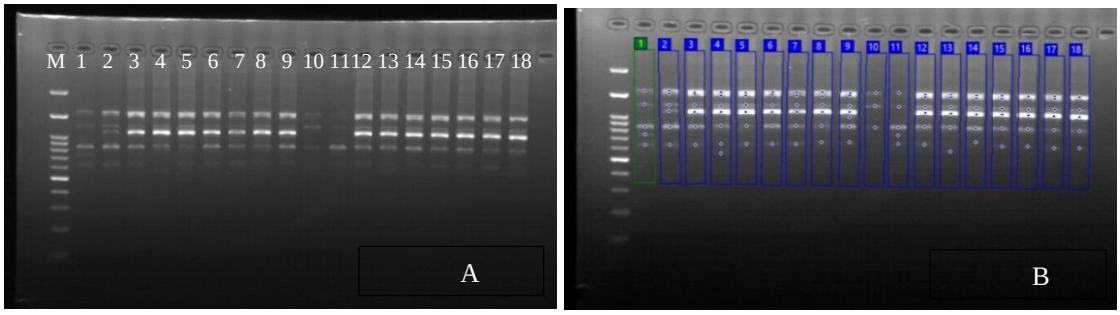


Figure 3.4. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 807 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 810: A total of 8 bands were amplified with primer ISSR 810 (Figure 3.5). The largest band size was 2231 bp and the smallest band was 467 bp, the polymorphism percentage of the primer was 87.5 % and the PIC was calculated as 0.2.

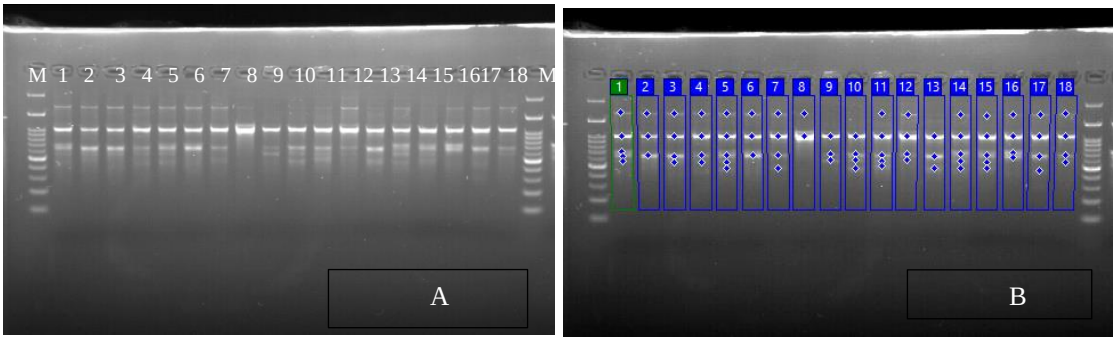


Figure 3.5. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 810 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 811: A total of 18 bands were amplified with primer ISSR 811 (Figure 3.6). The largest band size was 3919 bp and the smallest band was 333 bp, the polymorphism percentage of the primer was 94 % and the PIC was calculated as 0.26.

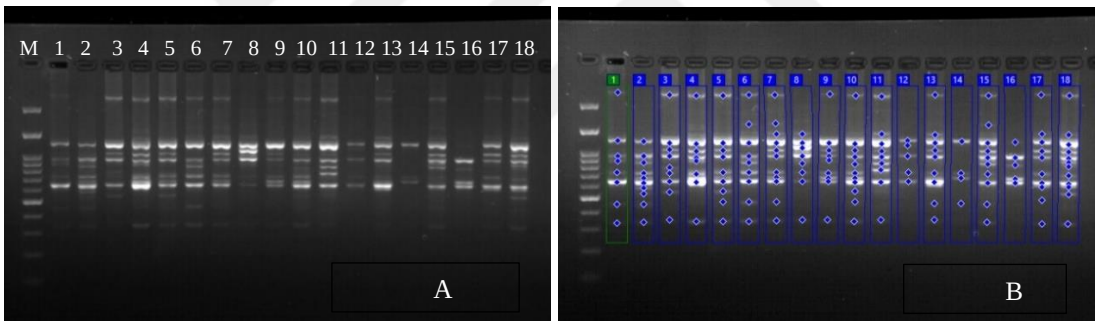


Figure 3.6. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 811 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 812: A total of 9 bands were amplified with primer ISSR 812 (Figure 3.7). The largest band size was 1700 bp and the smallest band was 560 bp, the polymorphism percentage of the primer was 67 % and PIC was calculated as 0.23.

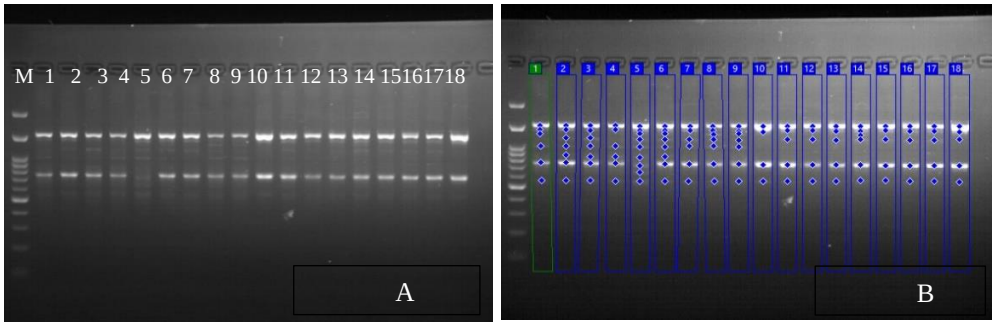


Figure 3.7. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 812 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 813: A total of 4 bands were amplified with primer ISSR 813 (Figure 3.8). The largest band size was 1560 bp and the smallest band was 406 bp, the polymorphism percentage of the primer was 50 % and PIC was calculated as 0.13.

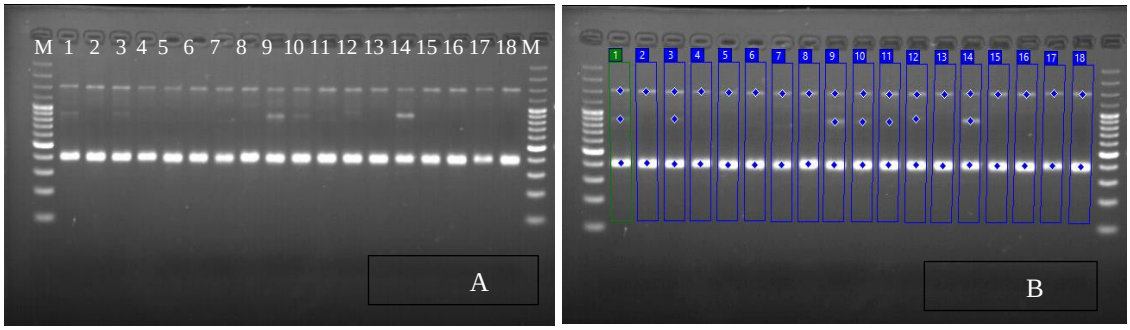


Figure 3.8. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 813 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 817: Only 2 monomorphic bands were amplified with primer ISSR 817 (Figure 3.9). The largest band size was 1320 bp and the smallest band was 949 bp, there is no polymorphism percentage or PIC of this primer.

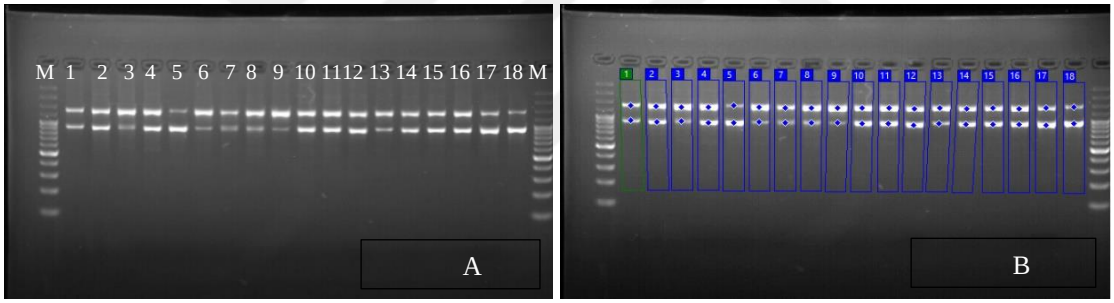


Figure 3.9. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 817 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 818: A total of 14 bands were amplified with primer ISSR 818 (Figure 3.10). The largest band size was 2381 bp and the smallest band was 609 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.3.

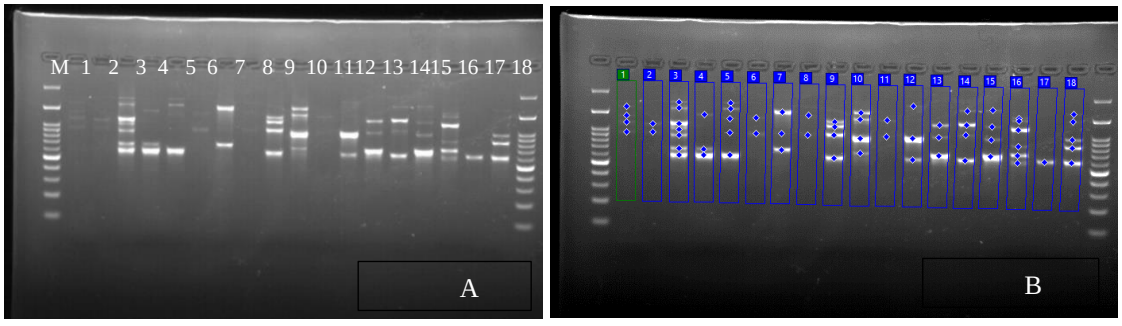


Figure 3.10. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 818 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 819: A total of 5 bands were amplified with primer ISSR 819 (Figure 3.11). The largest band size was 1423 bp and the smallest band was 712 bp, the polymorphism percentage of the primer was 60 % and PIC was calculated as 0.05.

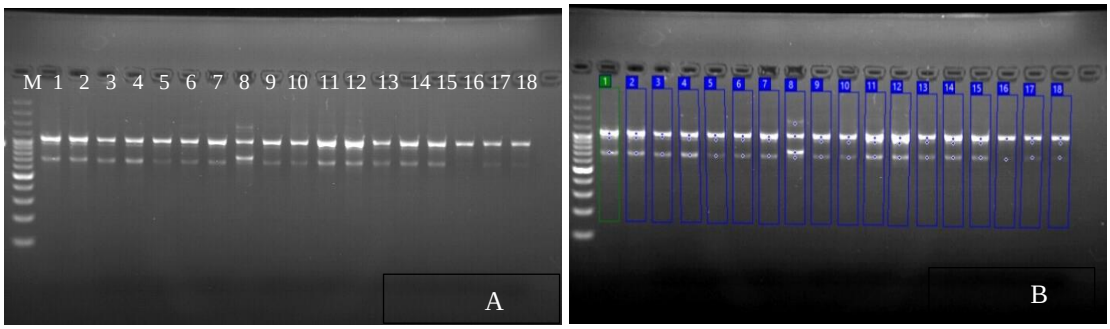


Figure 3.11. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 819 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 829: A total of 16 bands were amplified with primer ISSR 829 (Figure 3.12). The largest band size was 3000 bp and the smallest band was 510 bp, the polymorphism percentage of the primer was 94 % and PIC was calculated as 0.27.

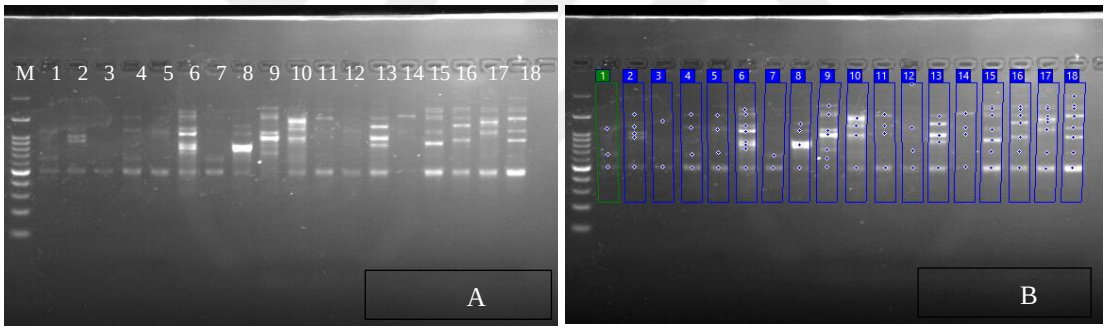


Figure 3.12. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 829 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 834: A total of 13 bands were amplified with primer ISSR 834 (Figure 3.13). The largest band size was 1122 bp and the smallest band was 364 bp, the polymorphism percentage of the primer was 92 % and PIC was calculated as 0.17.

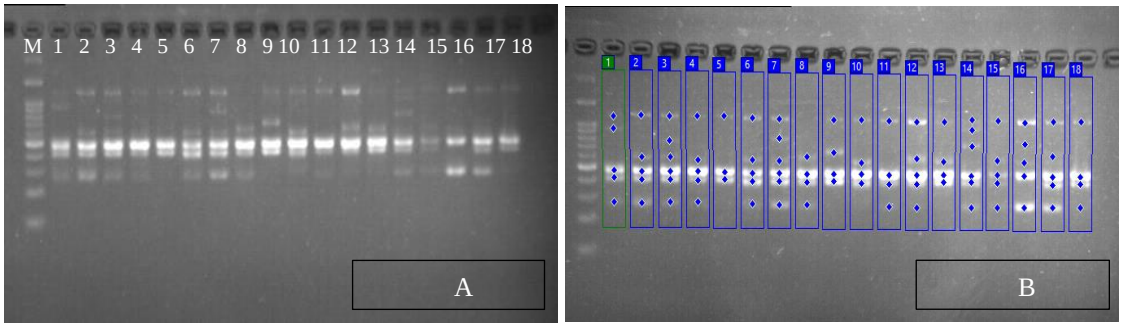


Figure 3.13. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 834 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 847: A total of 13 bands were amplified with primer ISSR 847 (Figure 3.14). The largest band size was 2368 bp and the smallest band was 582 bp, the polymorphism percentage of the primer was 77 % and PIC was calculated as 0.14.

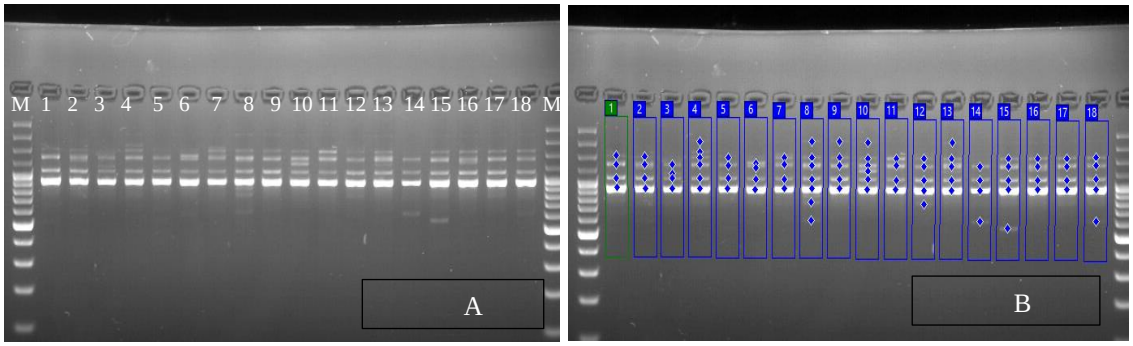


Figure 3.14. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 847 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

SSR 853: A total of 13 bands were amplified with primer ISSR 853 (Figure 3.15). The largest band size was 1433 bp and the smallest band was 400 bp, the polymorphism percentage of the primer was 77 % and PIC was calculated as 0.18.

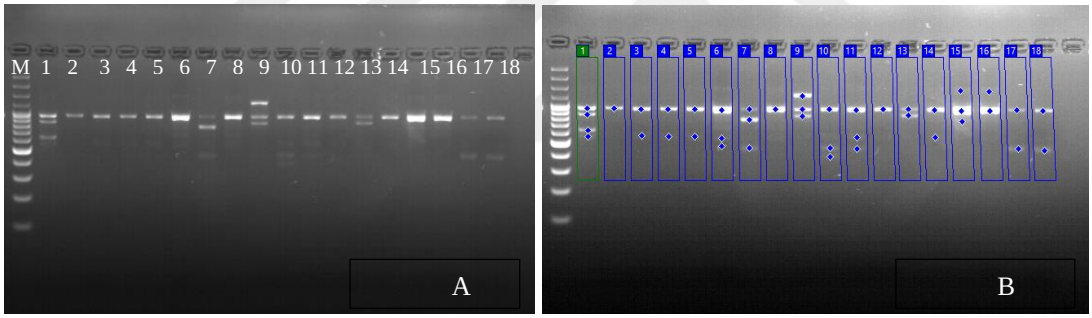


Figure 3.15. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 853 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

ISSR 855: A total of 10 bands were amplified with primer ISSR 855 (Figure 3.16). The largest band size was 1887 bp and the smallest band was 470 bp, the polymorphism percentage of the primer was 70 % and PIC was calculated as 0.1.

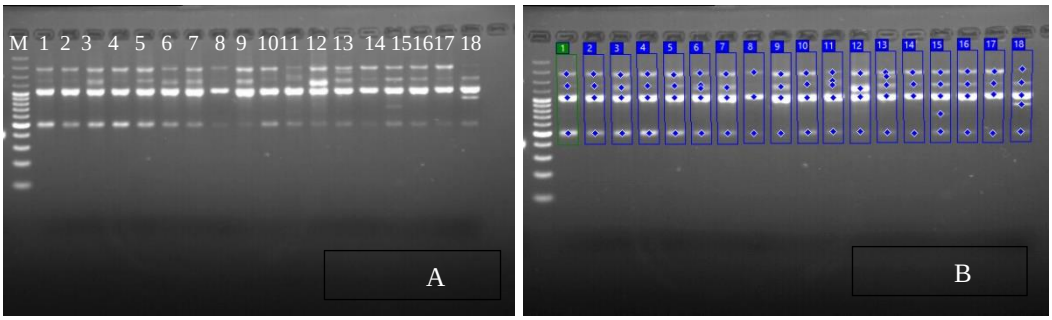


Figure 3.16. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer ISSR 855 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

3.4.2. ISSR-PCR analysis for the lentil accessions

13 ISSR primers were used to amplify 35 lentil accessions (27, *L. culinaris*; 8, *L. ervoides*) and their agarose gels were visualized under the UV light and digitally photographed (Figures 3.17- 3.29).

ISSR 807: A total of 12 bands were amplified with primer ISSR 807 (Figure 3.17). The largest band size was 2210 bp and the smallest band was 882 bp, the polymorphism percentage of the primer was 83 % and PIC was calculated as 0.19.

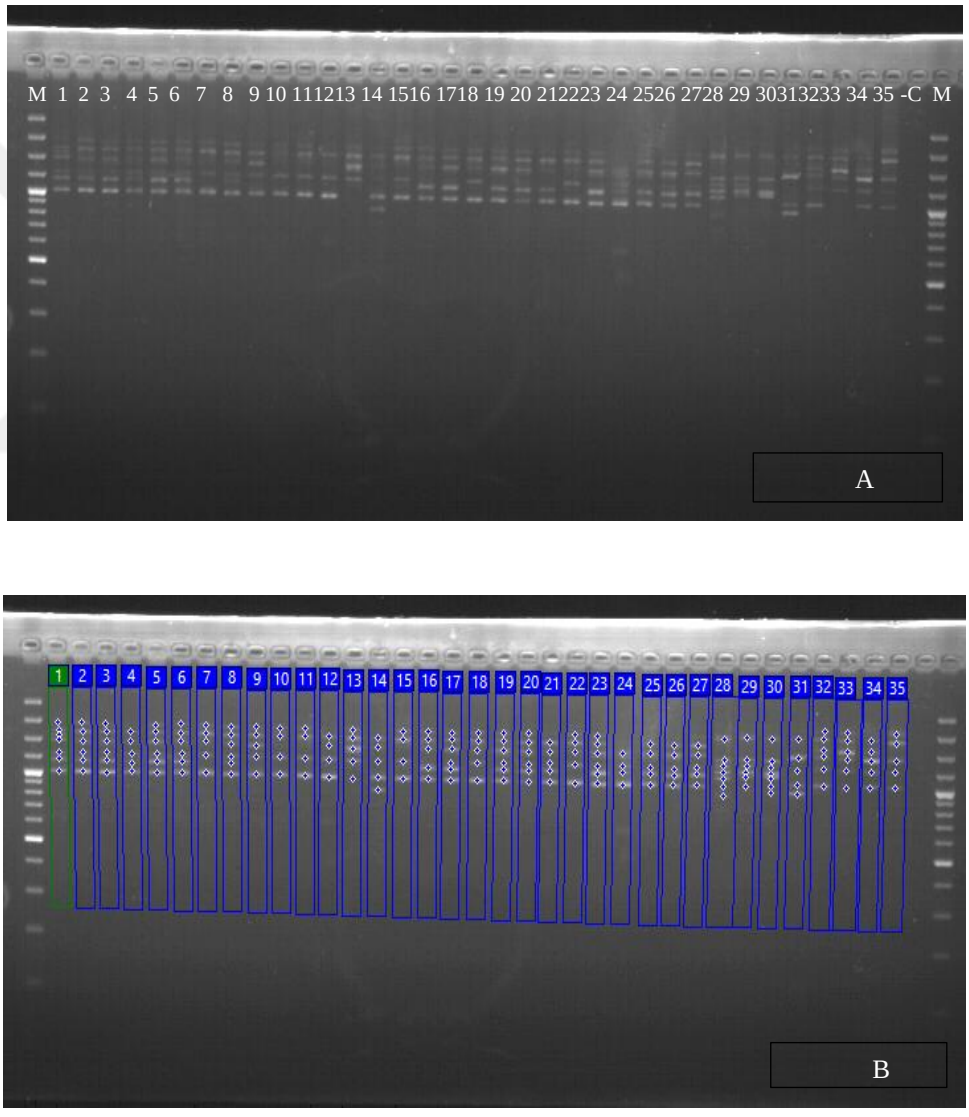


Figure 3.17. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 807 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 808: A total of 20 bands were amplified with primer ISSR 808 (Figure 3.18). The largest band size was 2088 bp and the smallest band was 423 bp, the polymorphism percentage of the primer was 95 % and PIC was calculated as 0.3.

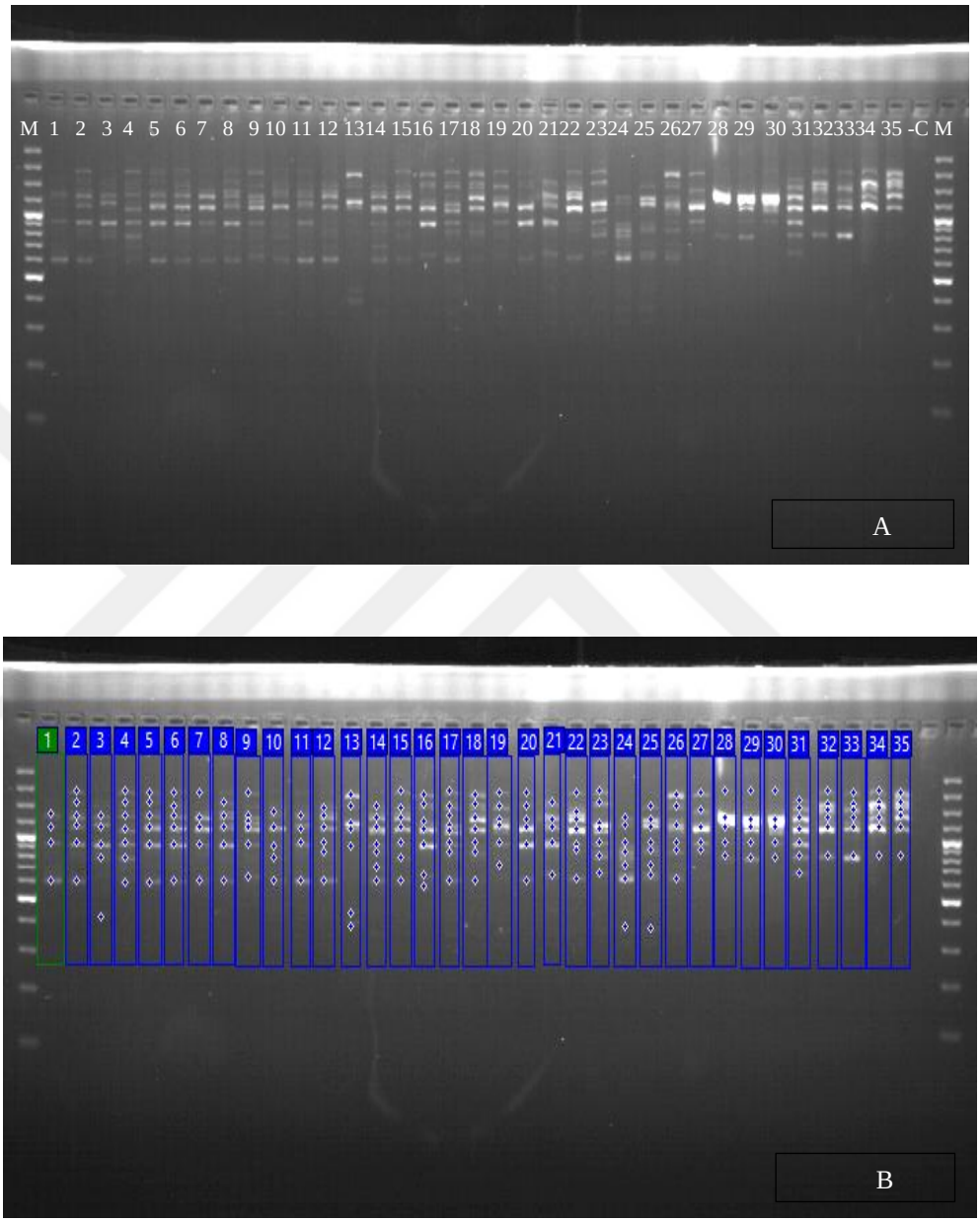


Figure 3.18. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 808 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 809: A total of 18 bands were amplified with primer ISSR 809 (Figure 3.19). The largest band size was 1737 bp and the smallest band was 454 bp, the polymorphism percentage of the primer was 89 % and PIC was calculated as 0.26.

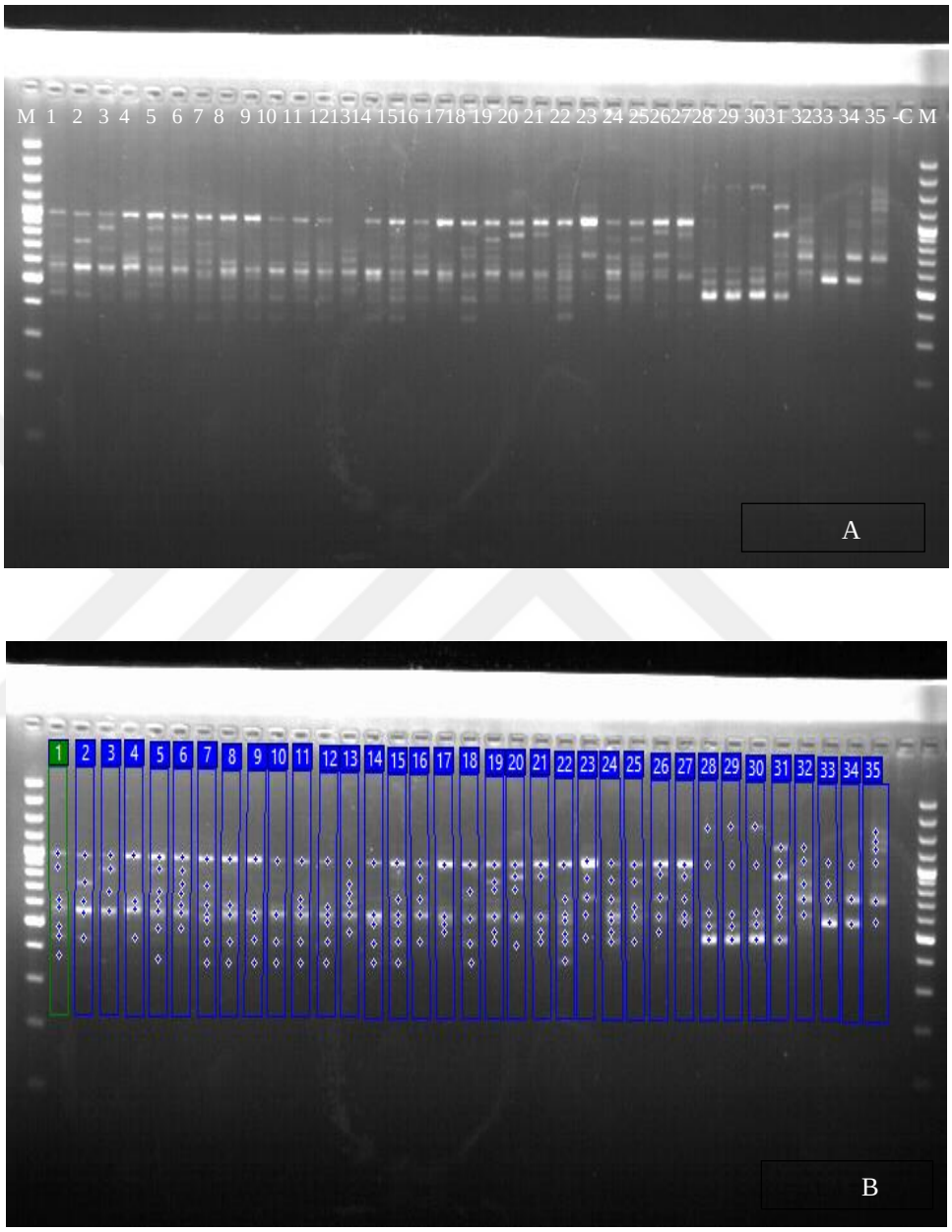


Figure 3.19. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 809 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 812: A total of 10 bands were amplified with primer ISSR 812 (Figure 3.20). The largest band size was 2288 bp and the smallest band was 858 bp, the polymorphism percentage of the primer was 90 % and PIC was calculated as 0.16.

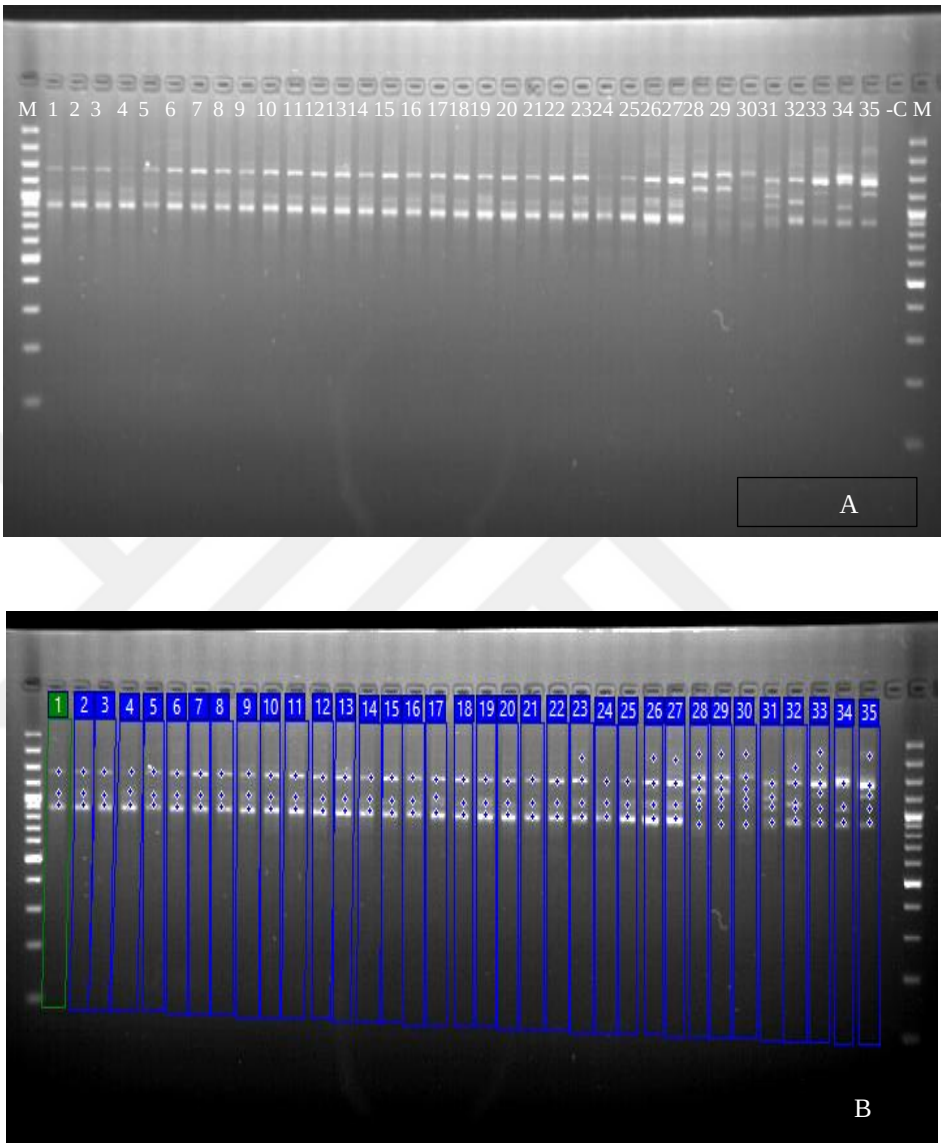


Figure 3.20. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 812 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 813: A total of 8 bands were amplified with primer ISSR 813 (Figure 3.21). The largest band size was 1619 bp and the smallest band was 700 bp, the polymorphism percentage of the primer was 87.5 % and PIC was calculated as 0.1.

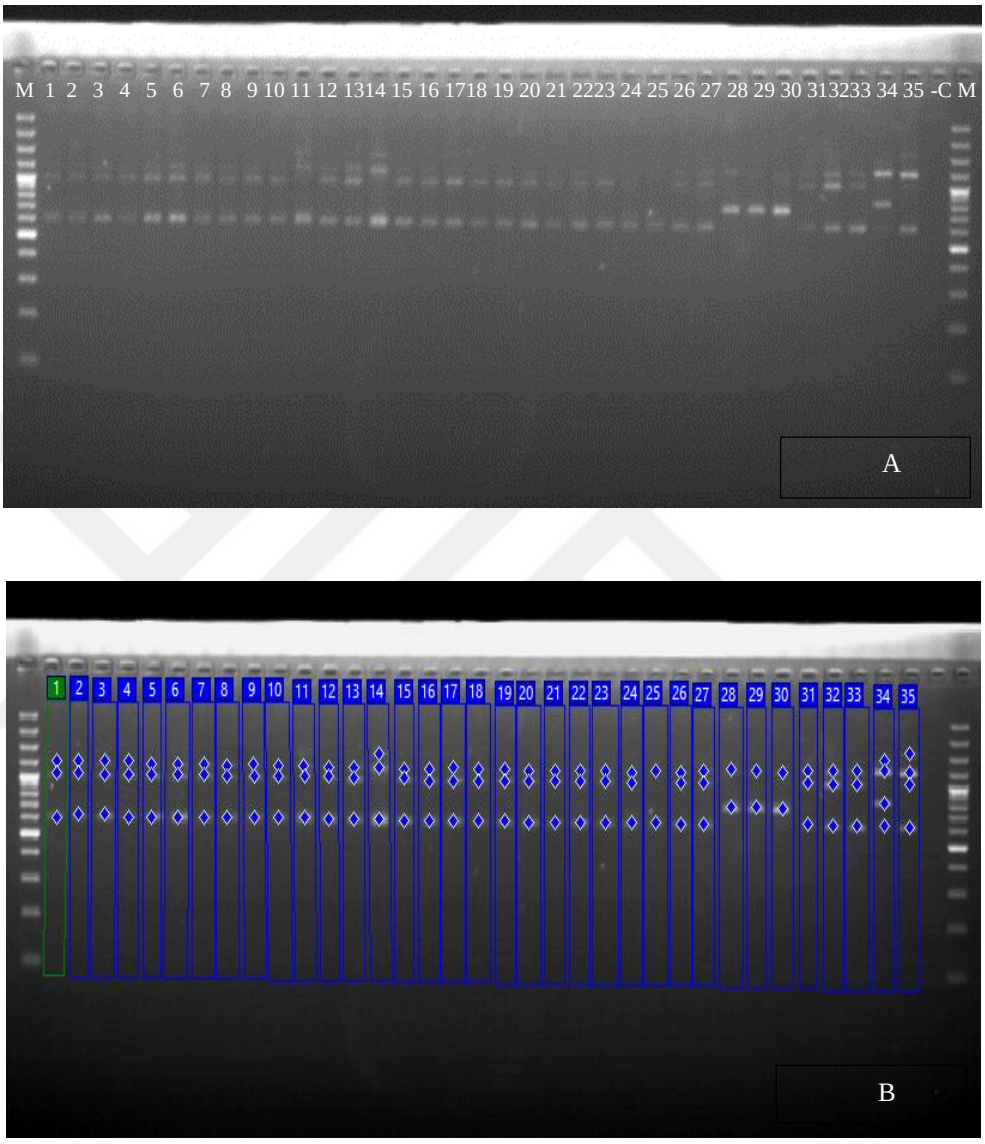


Figure 3.21. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 813 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 814: A total of 11 bands were amplified with primer ISSR 814 (Figure 3.22). The largest band size was 2000 bp and the smallest band was 570 bp, the polymorphism percentage of the primer was 91 % and PIC was calculated as 0.2.

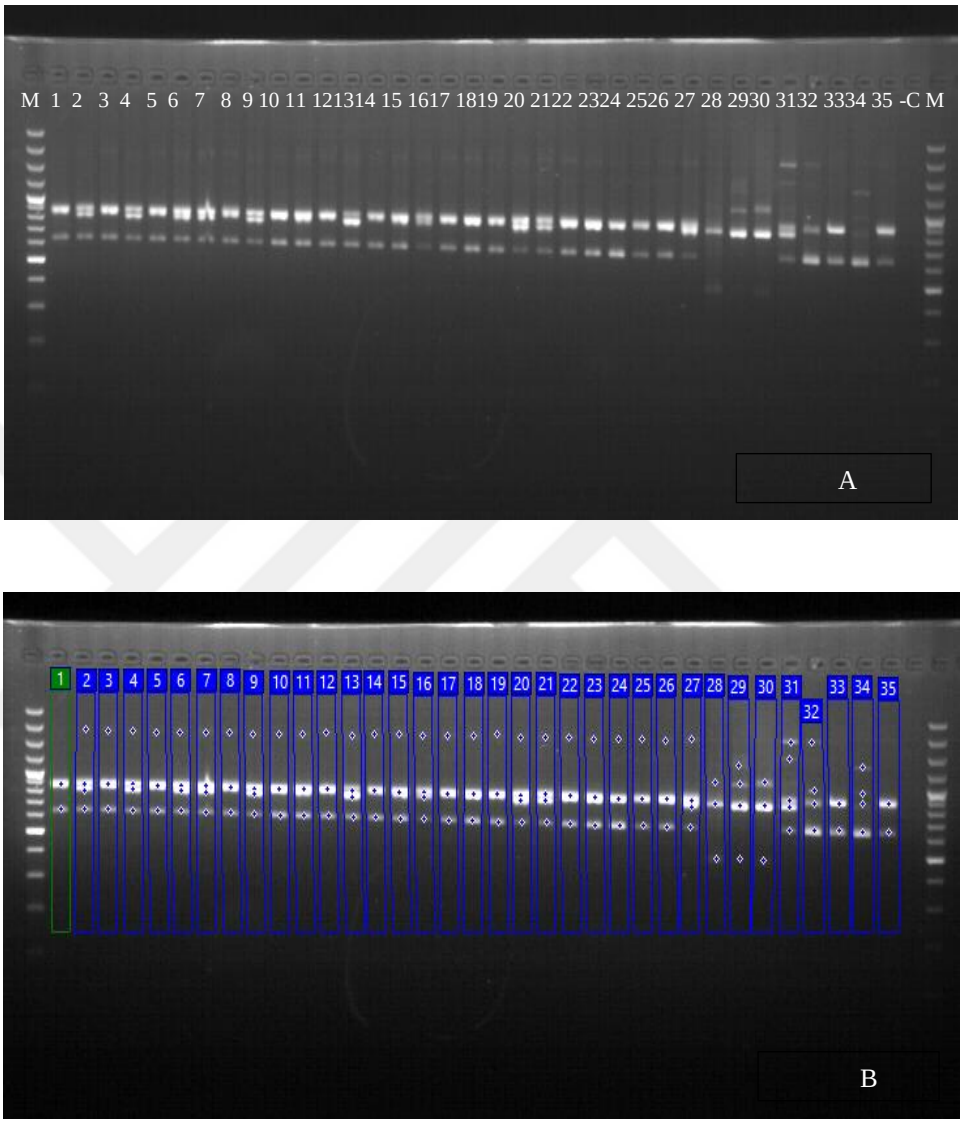


Figure 3.22. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 814 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 817: A total of 10 bands were amplified with primer ISSR 817 (Figure 3.23). The largest band size was 2460 bp and the smallest band was 800 bp, the polymorphism percentage of the primer was 90 % and PIC was calculated as 0.26.

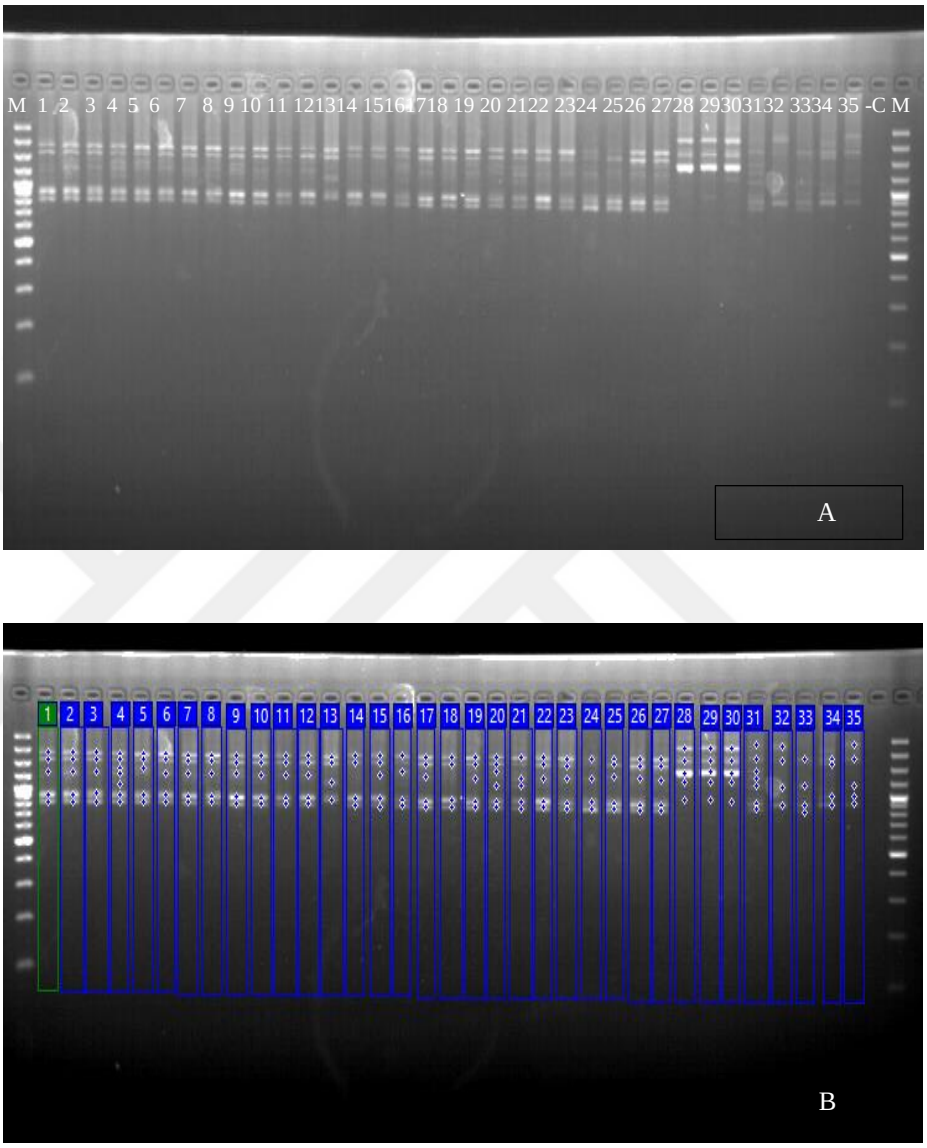


Figure 3.23. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 817 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 819: A total of 15 bands were amplified with primer ISSR 819 (Figure 3.24). The largest band size was 2887 bp and the smallest band was 611 bp, the polymorphism percentage of the primer was 93 % and PIC was calculated as 0.3.

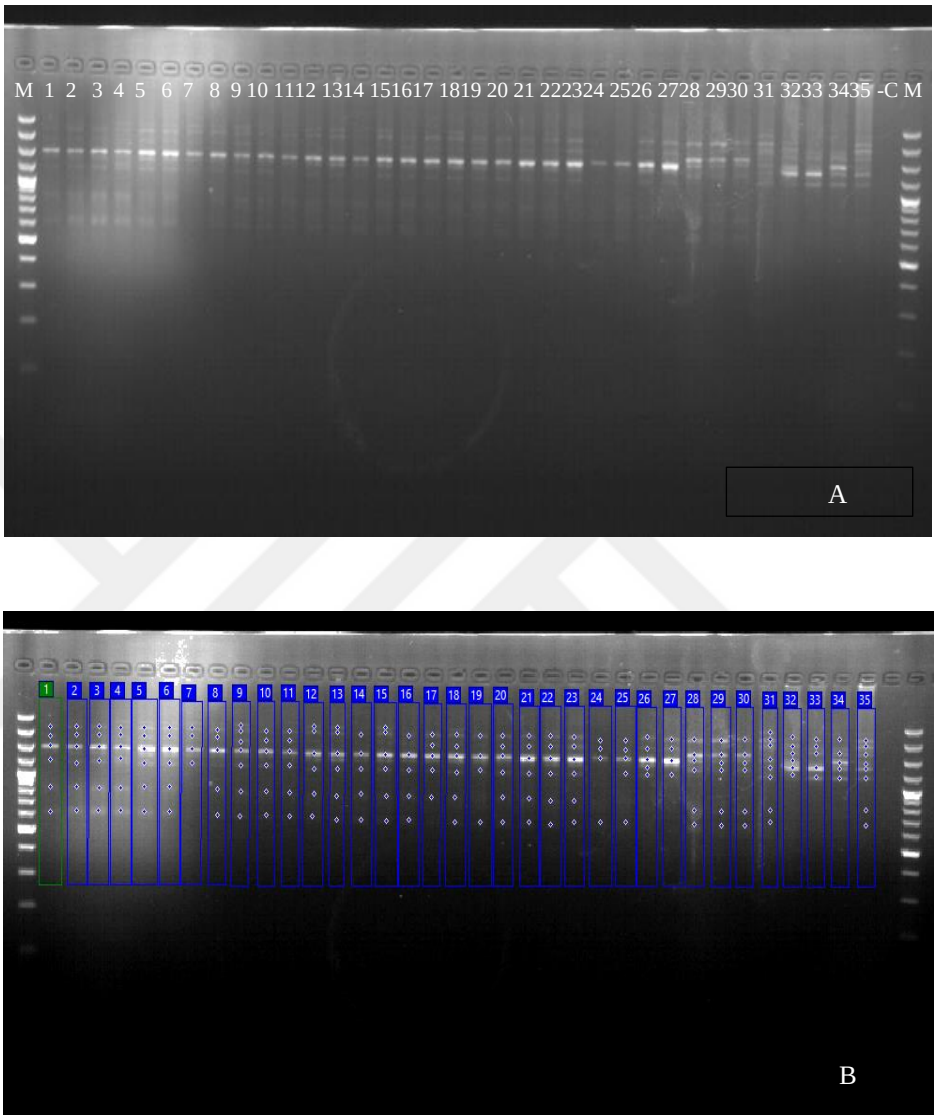


Figure 3.24. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 819 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 824: A total of 10 bands were amplified with primer ISSR 824 (Figure 3.25). The largest band size was 1640 bp and the smallest band was 391 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.3.

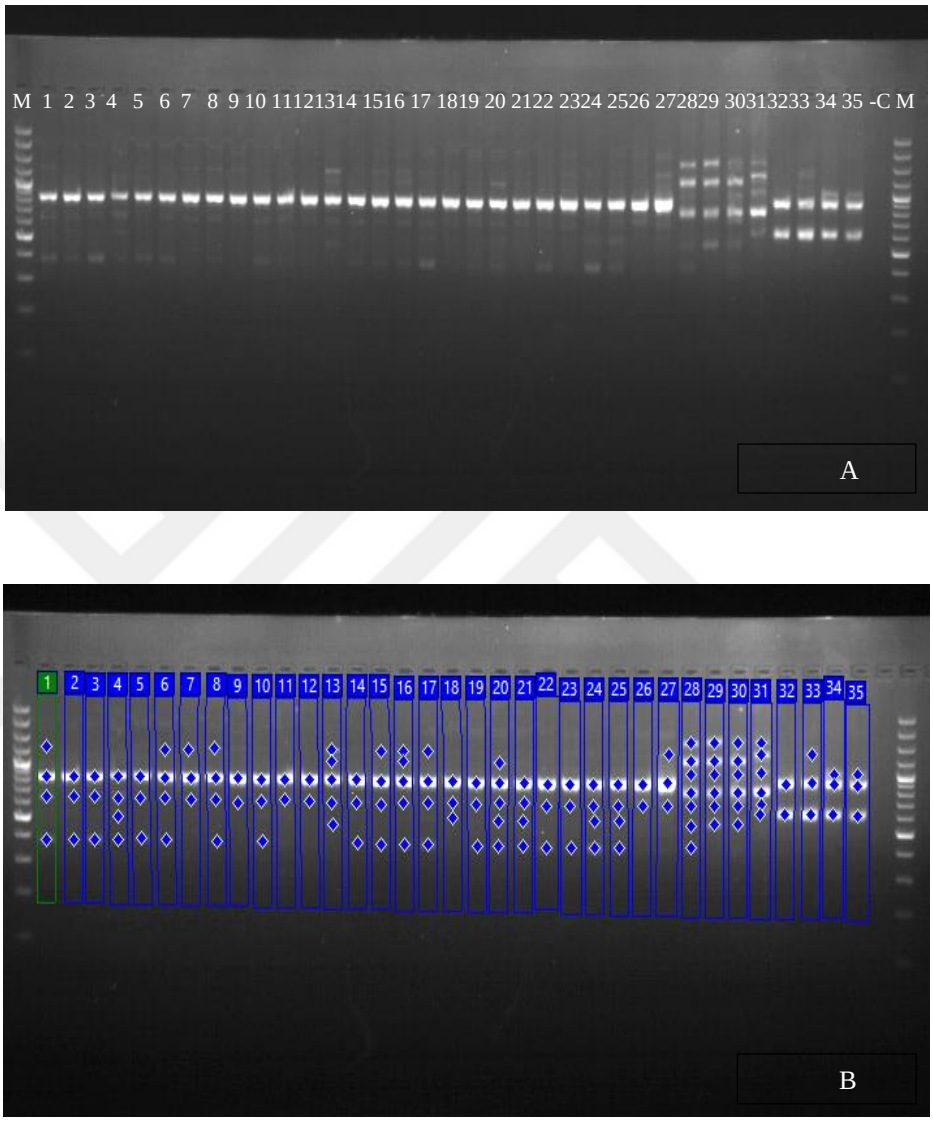


Figure 3.25. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 824 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 834: A total of 15 bands were amplified with primer ISSR 834 (Figure 3.26). The largest band size was 2209 bp and the smallest band was 306 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.31.

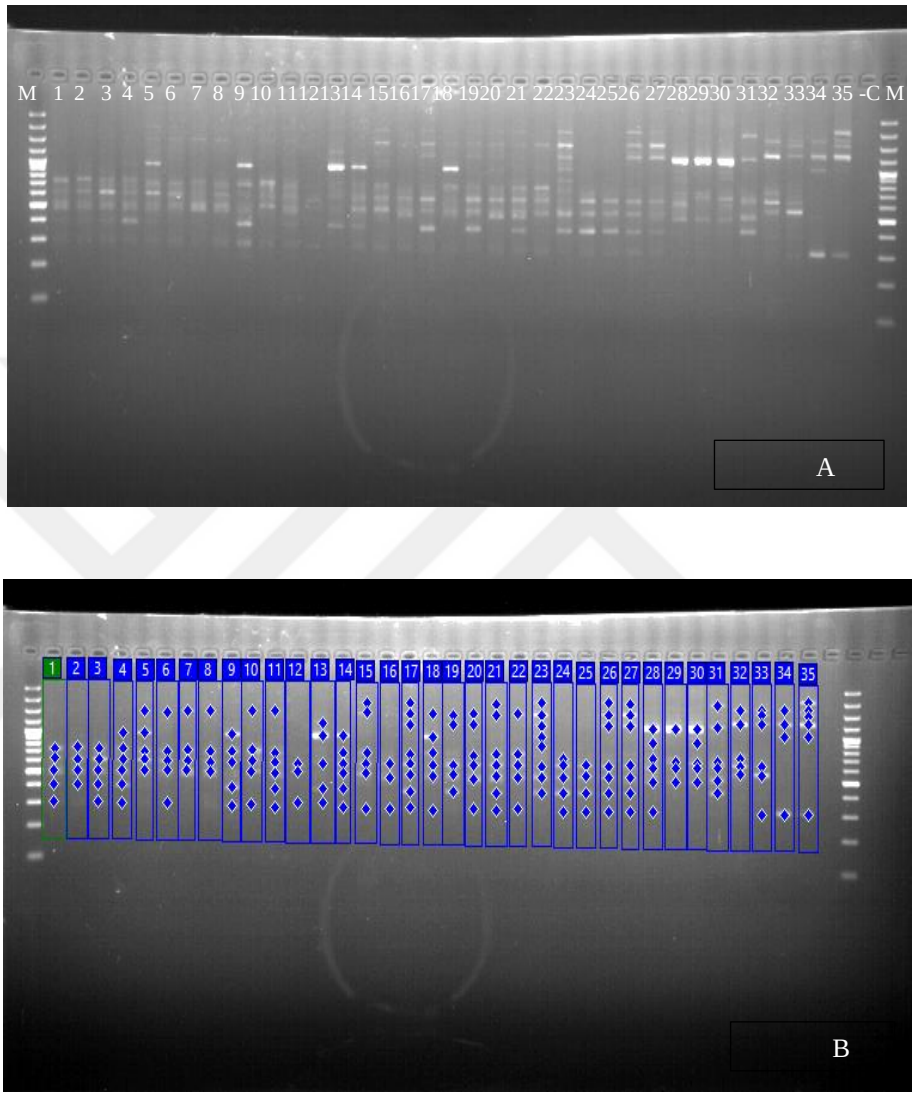


Figure 3.26. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 834 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 847: A total of 16 bands were amplified with primer ISSR 847 (Figure 3.27). The largest band size was 2160 bp and the smallest band was 563 bp, the polymorphism percentage of the primer was 94 % and PIC was calculated as 0.21.

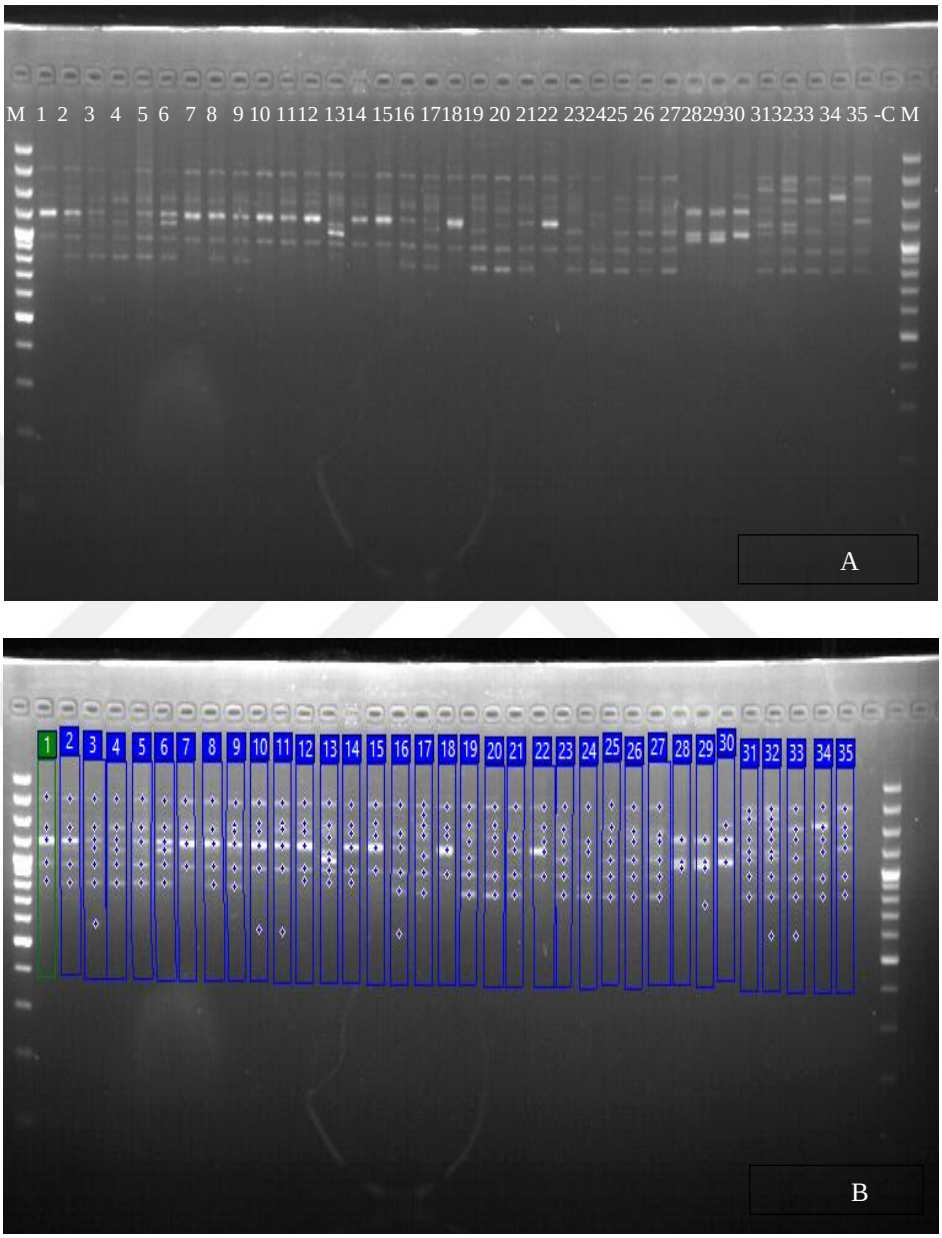


Figure 3.27. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 847 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 853: A total of 11 bands were amplified with primer ISSR 853 (Figure 3.28). The largest band size was 1739 bp and the smallest band was 534 bp, the polymorphism percentage of the primer was 91 % and PIC was calculated as 0.25.

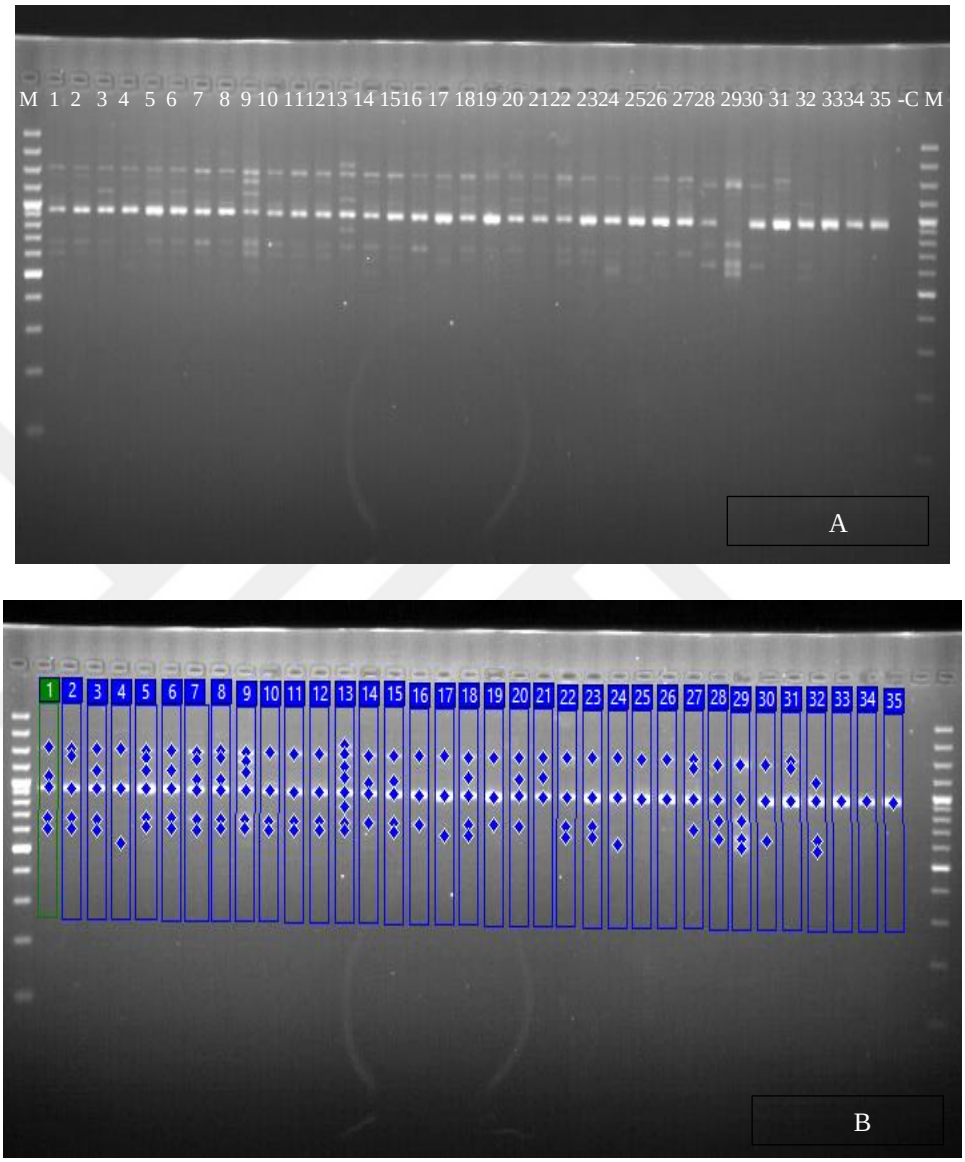


Figure 3.28. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 853 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 855: A total of 14 bands were amplified with primer ISSR 855 (Figure 3.29). The largest band size was 2412 bp and the smallest band was 611 bp, the polymorphism percentage of the primer was 93 % and PIC was calculated as 0.33.

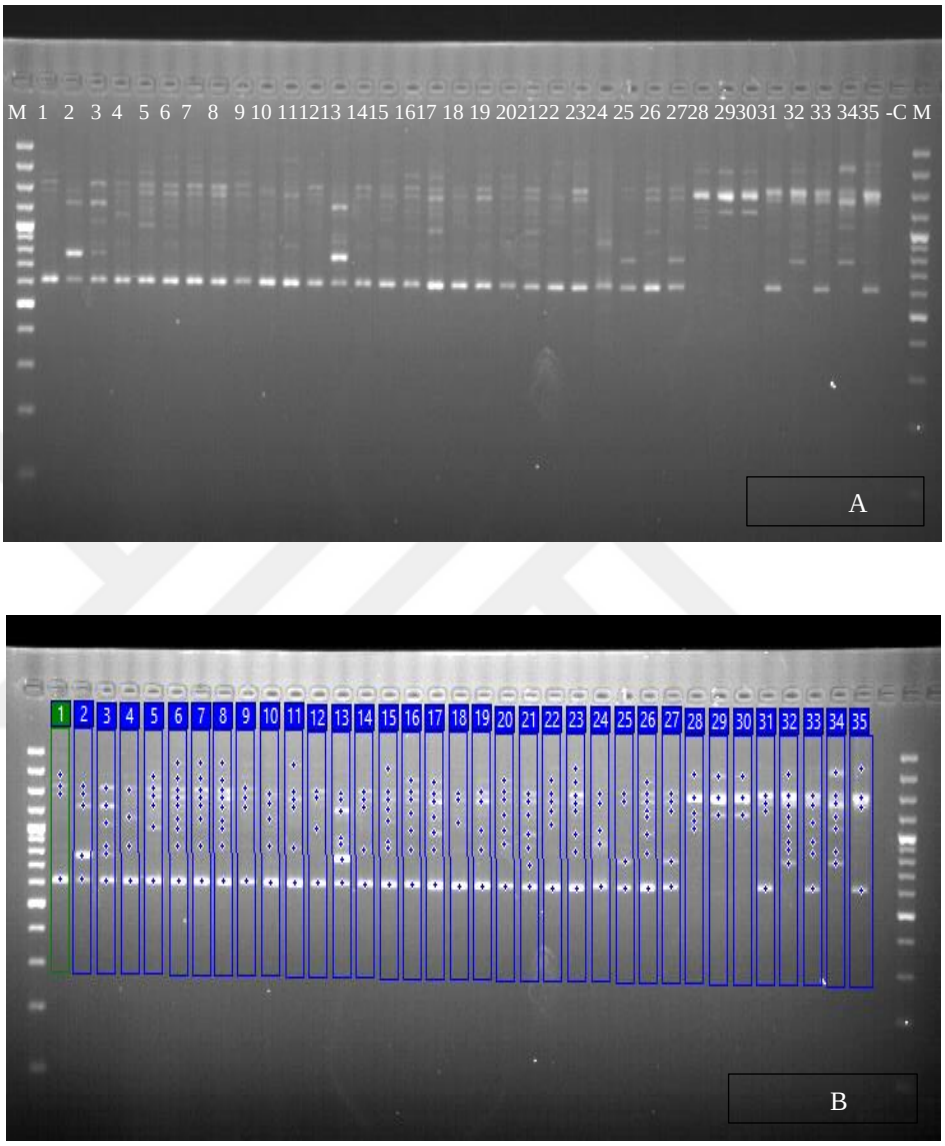


Figure 3.29. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer ISSR 855 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

3.4.3. ISSR-PCR analysis for the almond accessions

A total of 13 ISSR primers were used to amplify 36 almond accessions (24, cultivated; 12, wild). PCR products in agarose gels were visualized under the UV light and digitally photographed (Figures 3.30- 3.42).

ISSR 807: A total of 17 bands were amplified with primer ISSR 807 (Figure 3.30). The largest band size was 2256 bp and the smallest band was 366 bp, the polymorphism percentage of the primer was 94 % and PIC was calculated as 0.25.

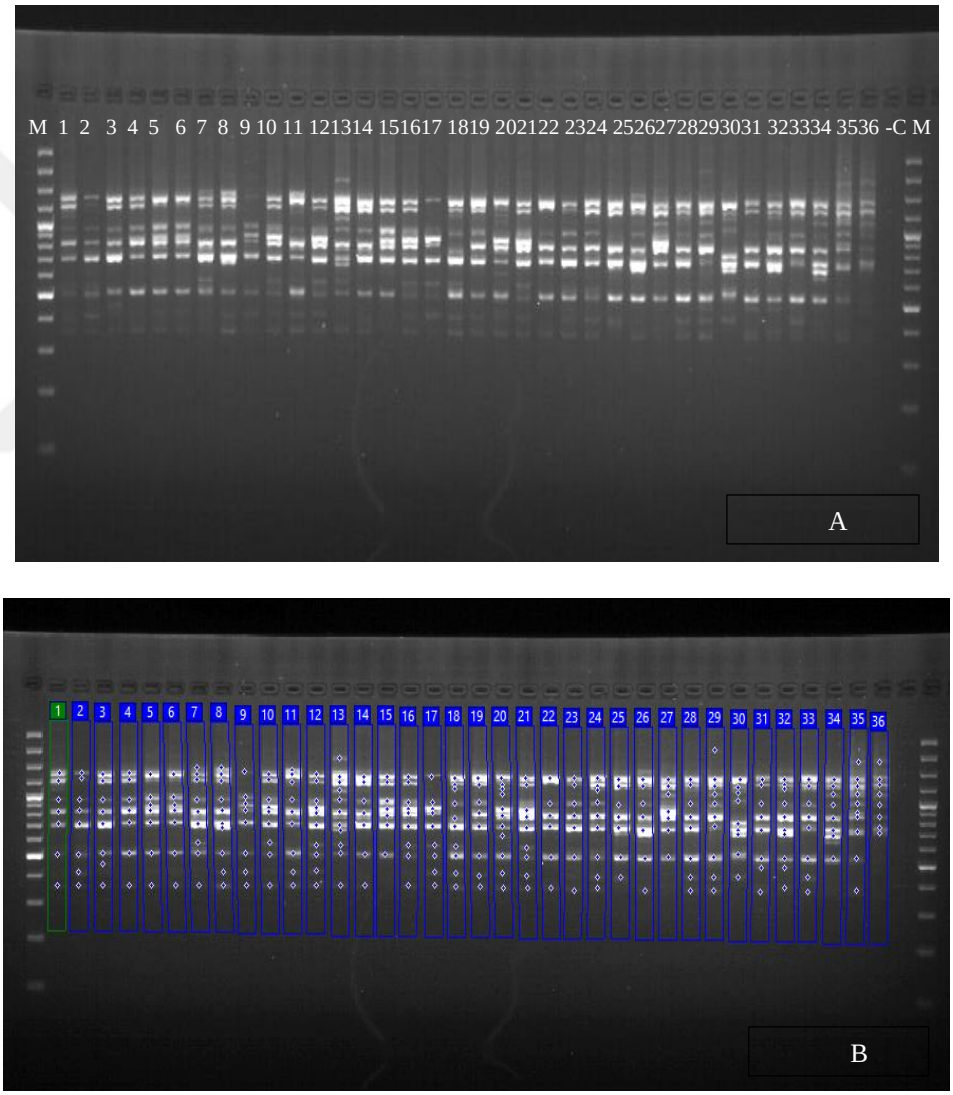


Figure 3.30. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 807 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 808: A total of 15 bands was amplified with primer ISSR 808 (Figure 3.31). The largest band size was 1927 bp and the smallest band was 500 bp, the polymorphism percentage of the primer was 87 % and PIC was calculated as 0.23.

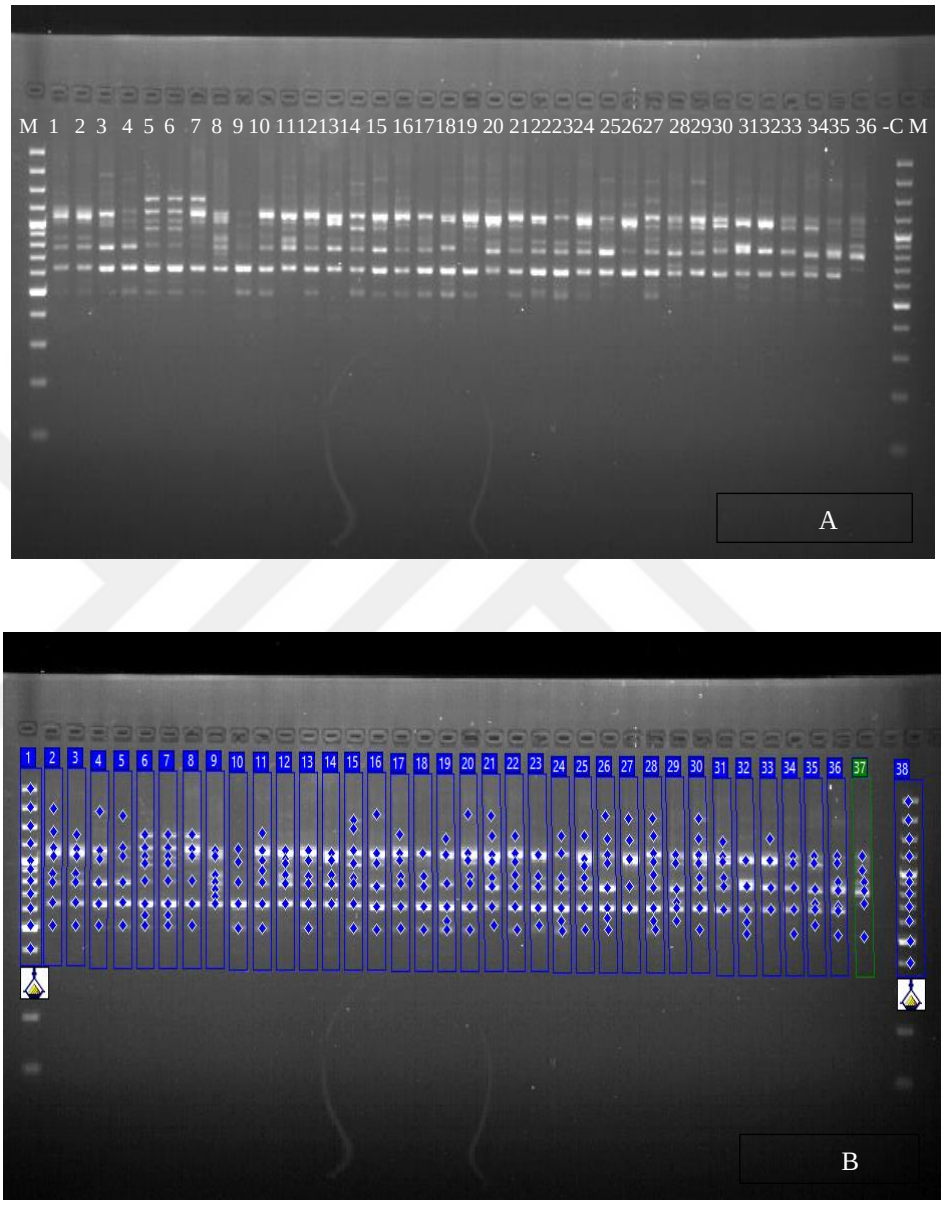


Figure 3.31. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 808 (A). Band detected with TotalLab Cliqs program (2-37) (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 809: A total of 14 bands was amplified with primer ISSR 809 (Figure 3.32). The largest band size was 1500 bp and the smallest band was 425 bp, the polymorphism percentage of the primer was 93 % and PIC was calculated as 0.22.

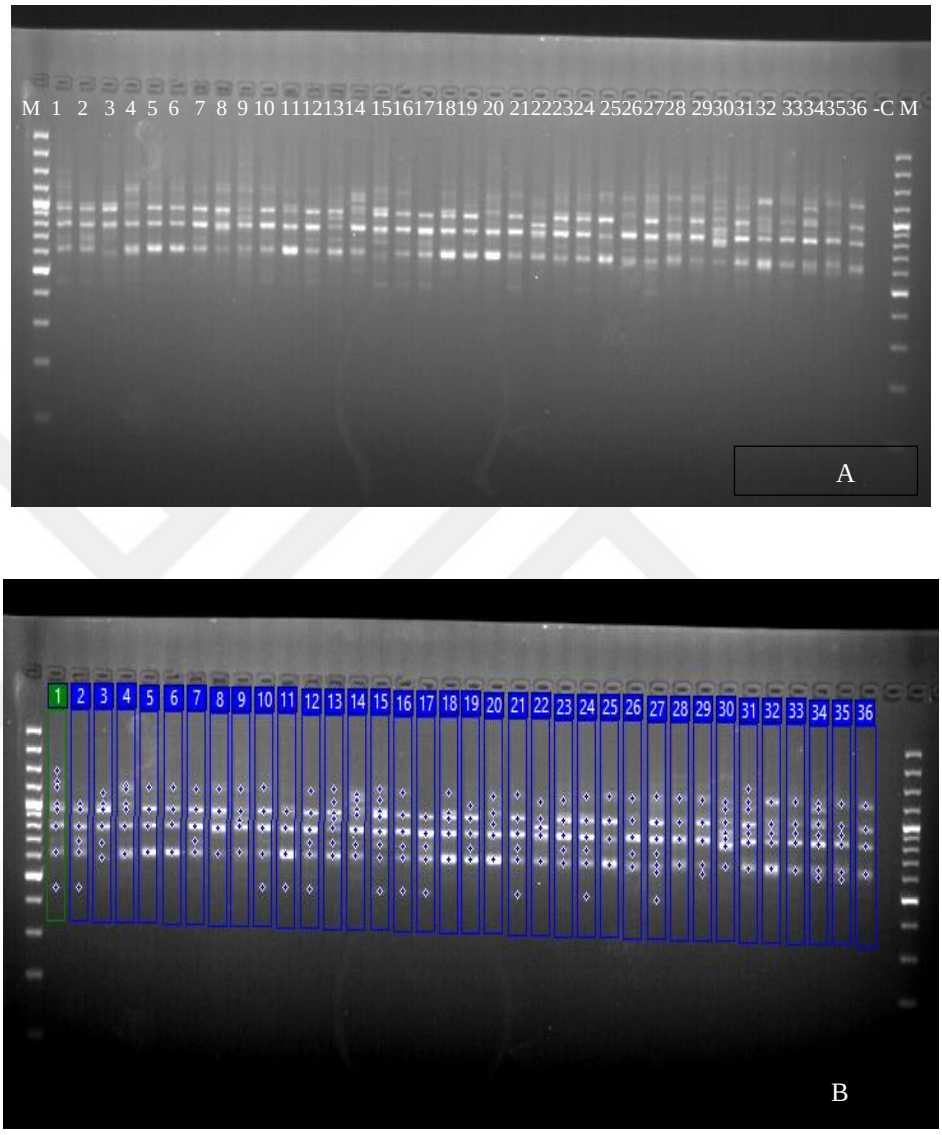


Figure 3.32. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 809 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 810: A total of 14 bands was amplified with primer ISSR 810 (Figure 3.33). The largest band size was 2511 bp and the smallest band was 535 bp, the polymorphism percentage of the primer was 93 % and PIC was calculated as 0.24.

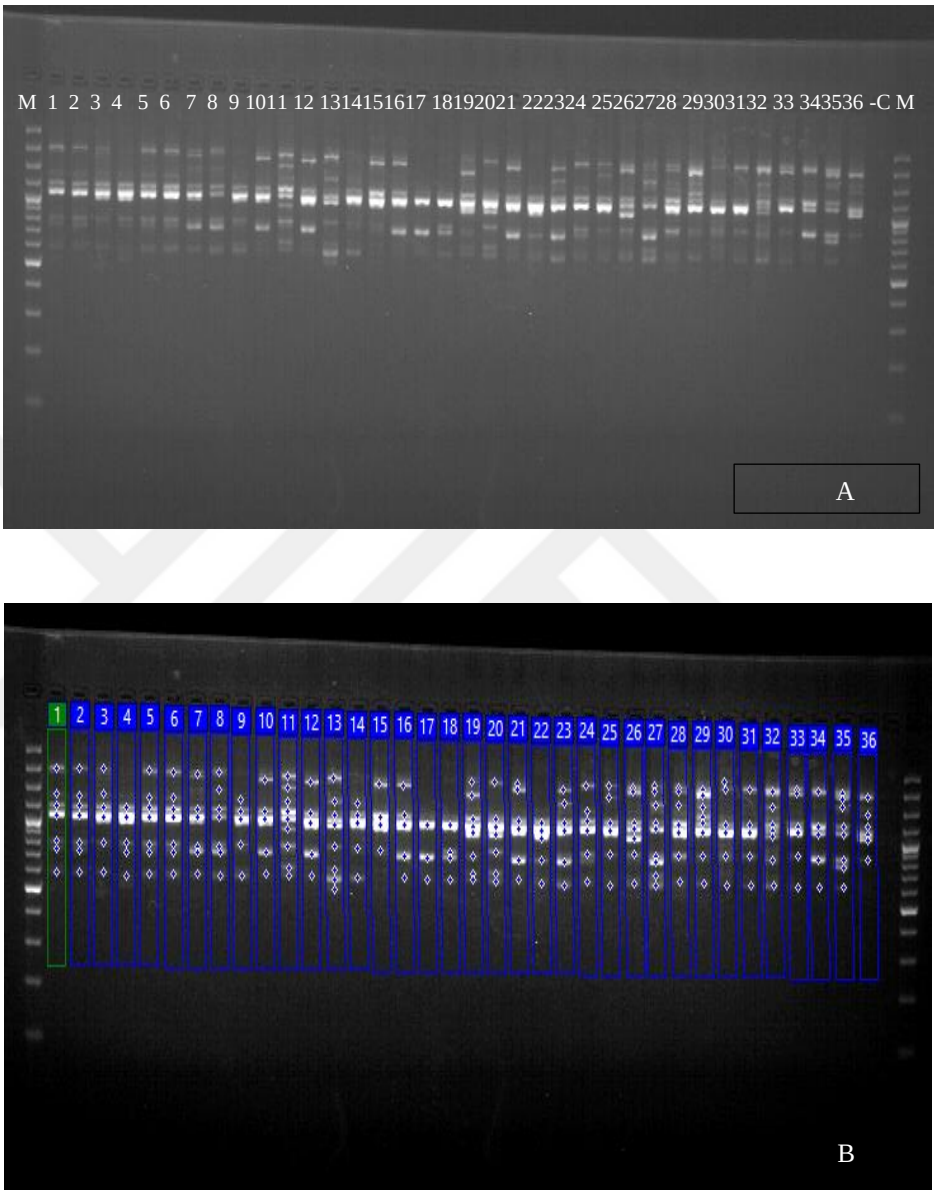


Figure 3.33. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 810 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 814: A total of 13 bands was amplified with primer ISSR 814 (Figure 3.34). The largest band size was 2000 bp and the smallest band was 509 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.28.

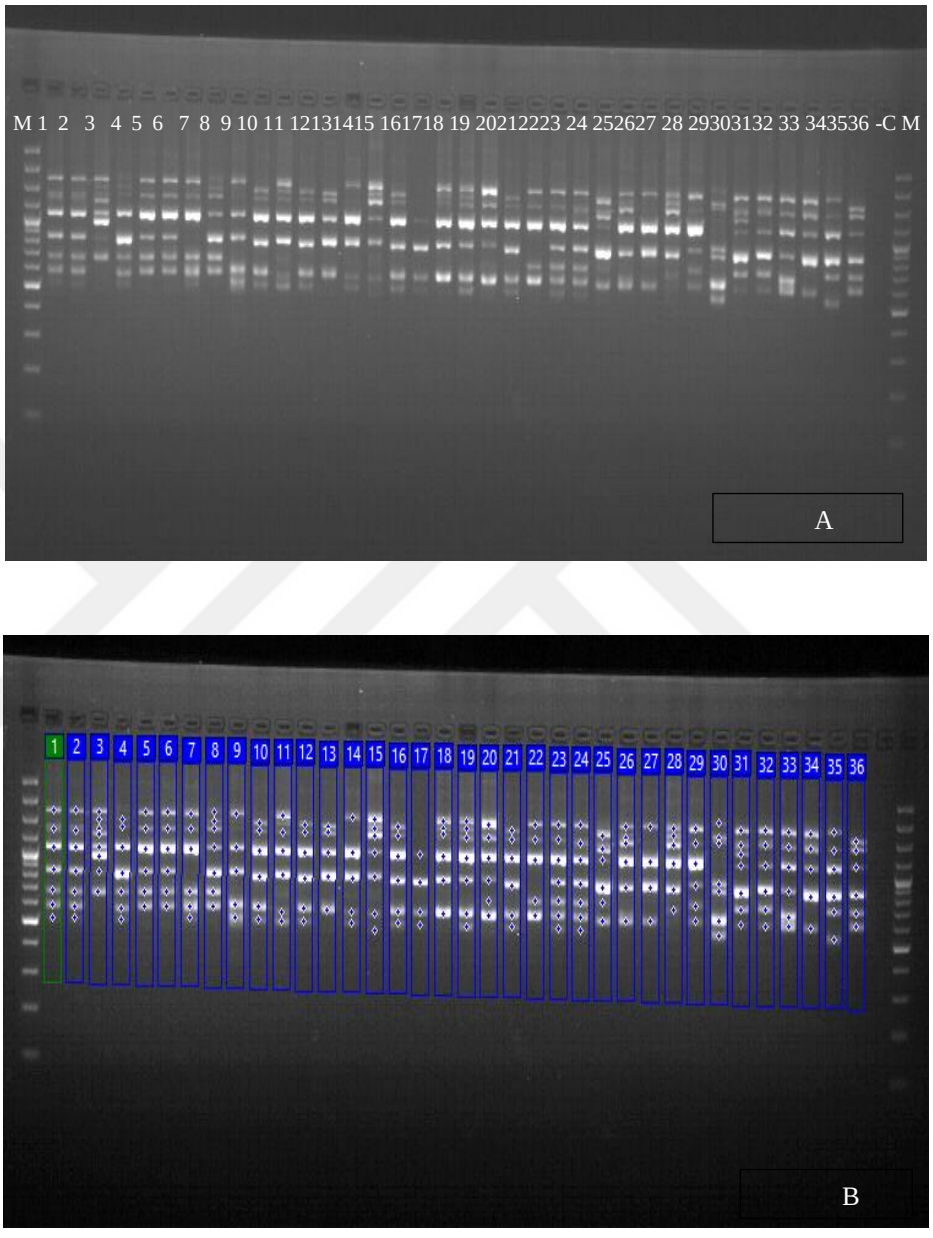


Figure 3.34. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 814 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 815: A total of 11 bands was amplified with primer ISSR 815 (Figure 3.35). The largest band size was 1767 bp and the smallest band was 543 bp, the polymorphism percentage of the primer was 82 % and PIC was calculated as 0.21.

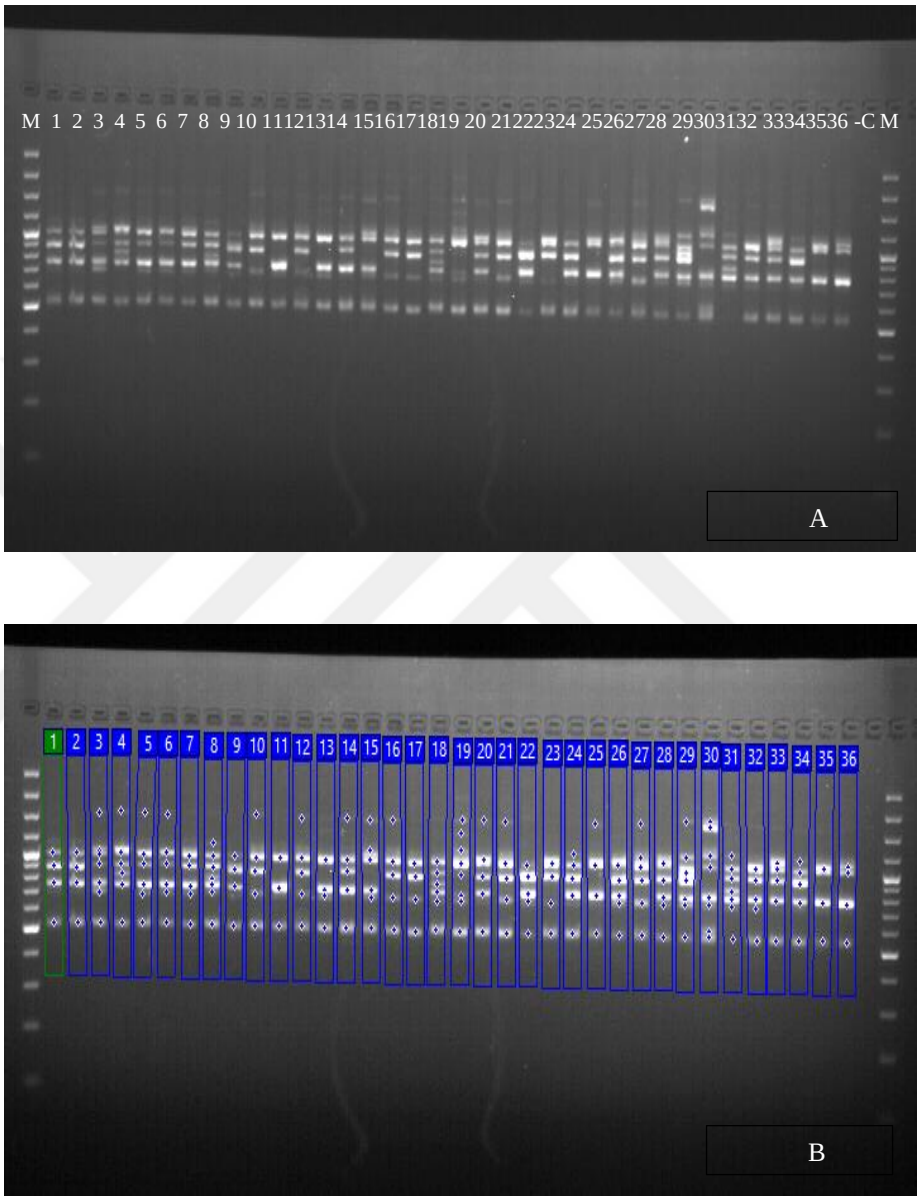


Figure 3.35. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 815 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 817: A total of 10 bands was amplified with primer ISSR 817 (Figure 3.36). The largest band size was 2000 bp and the smallest band was 733 bp, the polymorphism percentage of the primer was 80 % and PIC was calculated as 0.23.

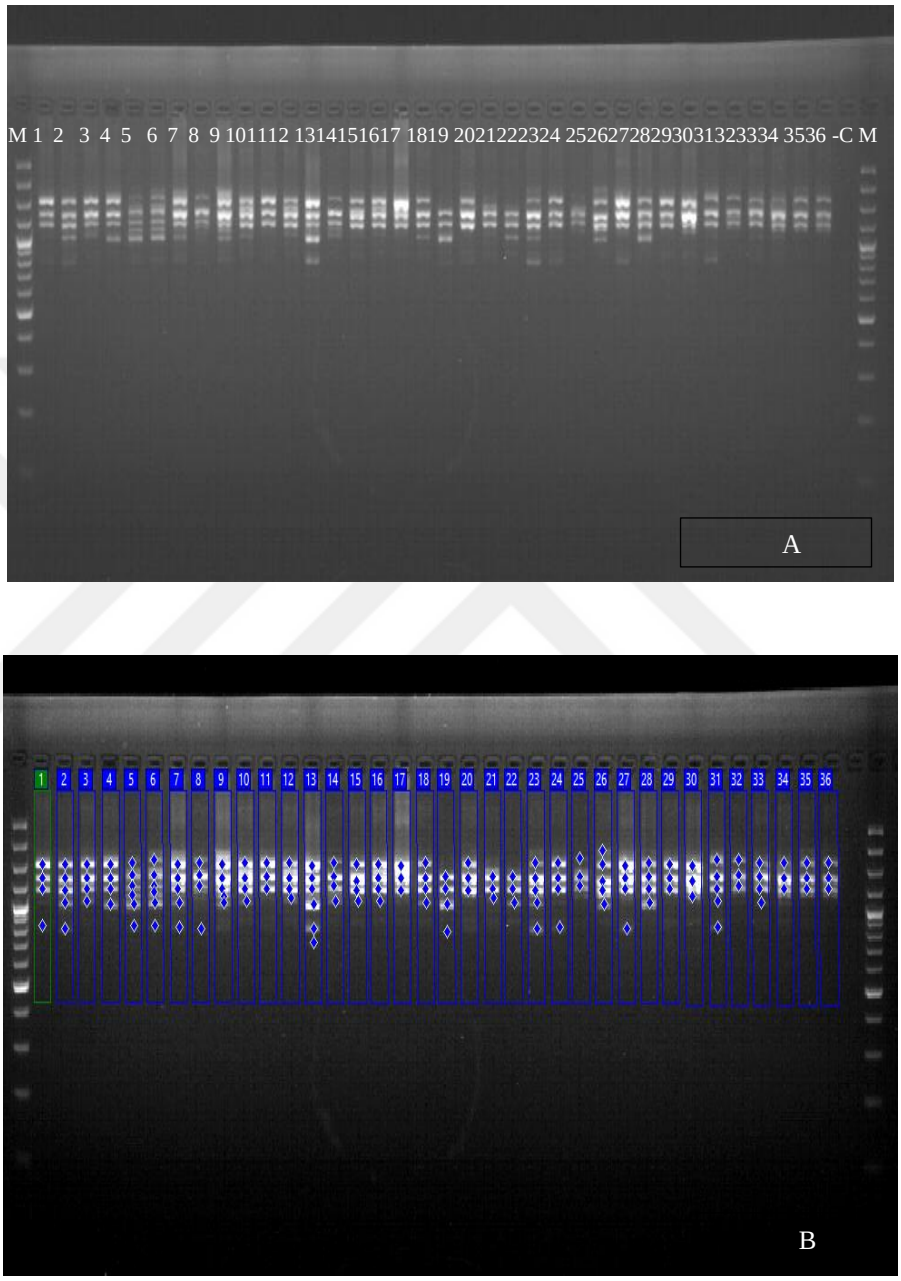


Figure 3.36. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 817 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 829: A total of 13 bands was amplified with primer ISSR 829 (Figure 3.37). The largest band size was 1305 bp and the smallest band was 321 bp, the polymorphism percentage of the primer was 92 % and PIC was calculated as 0.22.

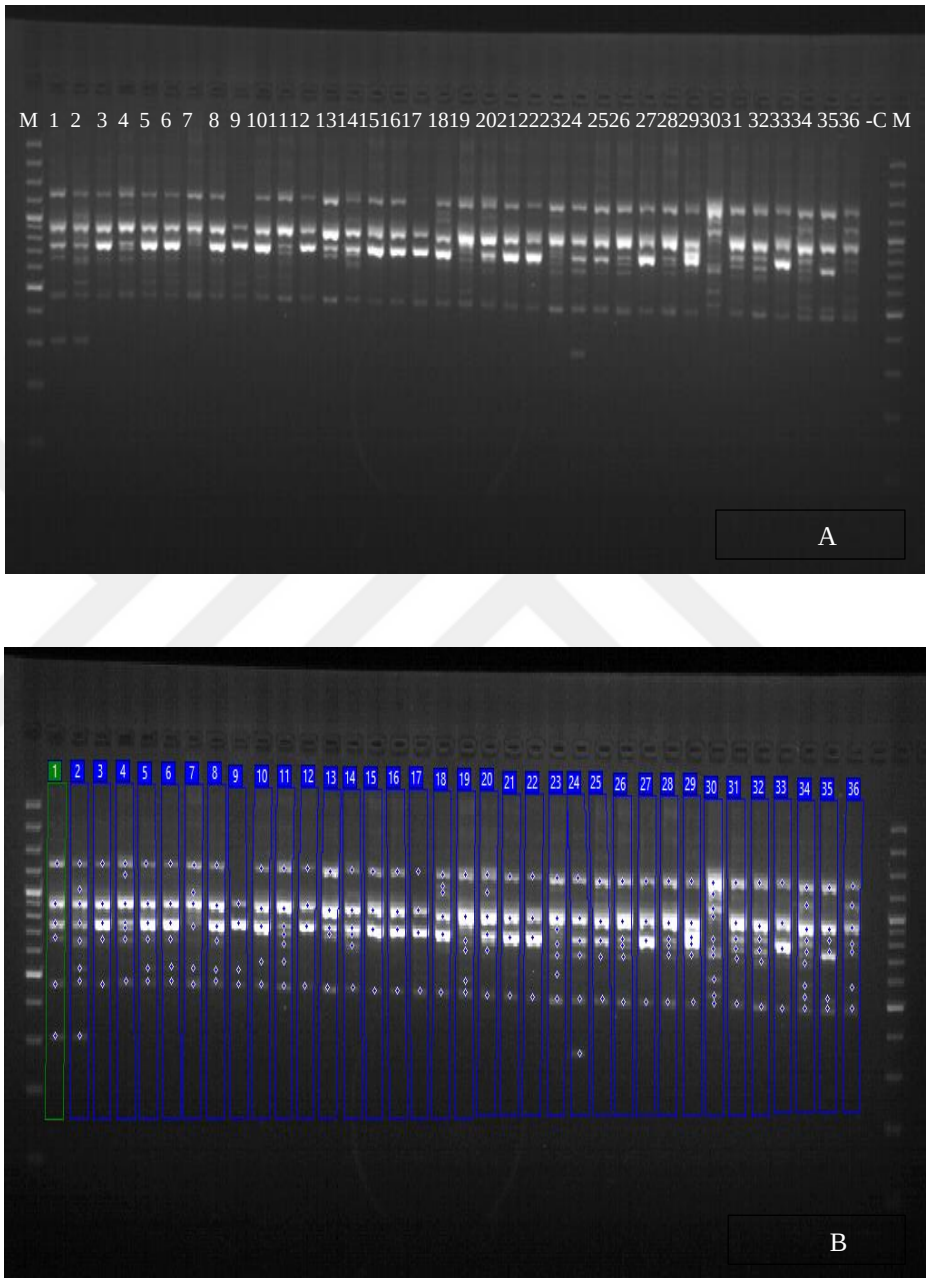


Figure 3.37. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 829 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 834: A total of 20 bands was amplified with primer ISSR 834 (Figure 3.38). The largest band size was 2627 bp and the smallest band was 294 bp, the polymorphism percentage of the primer was 95 % and PIC was calculated as 0.23.

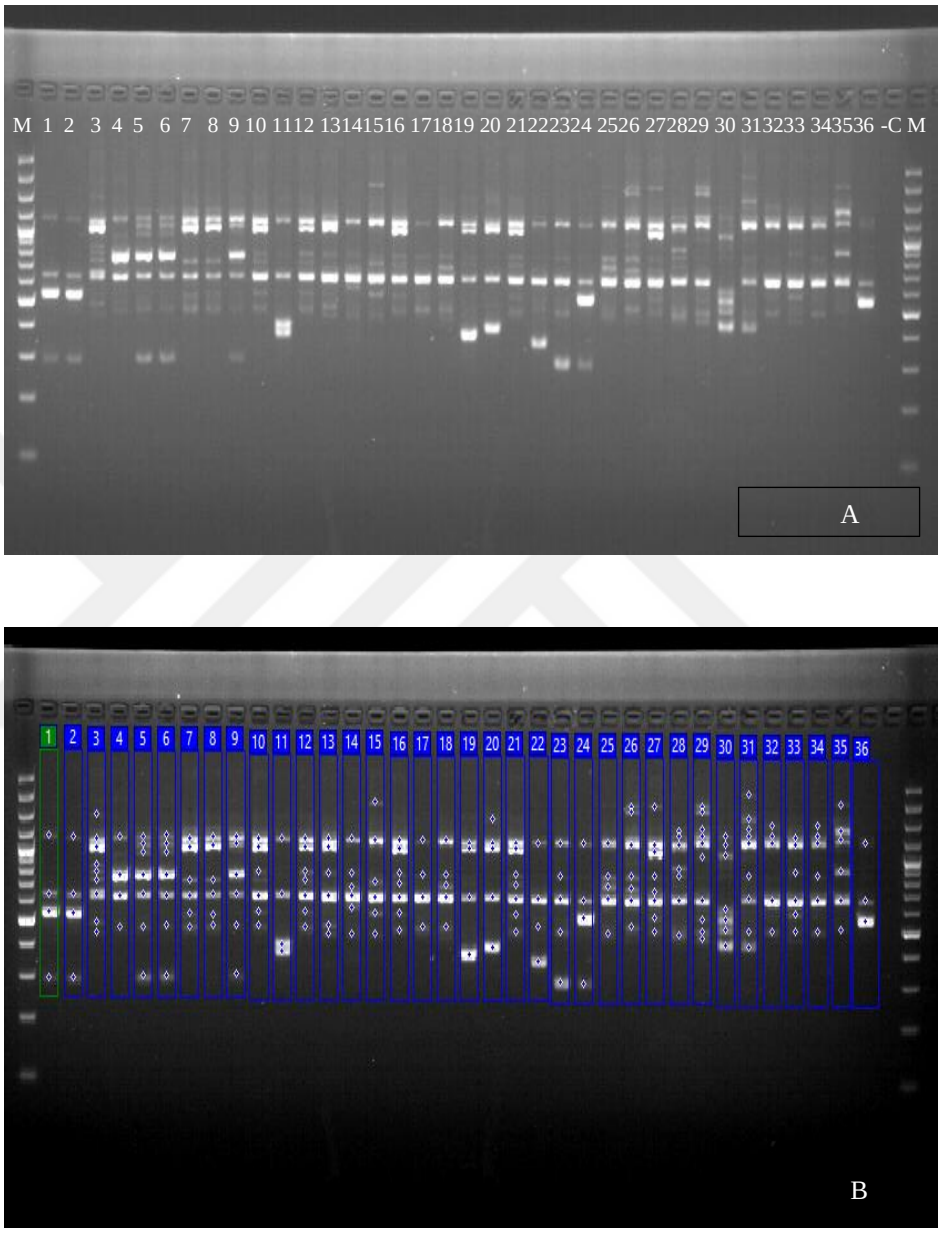


Figure 3.38. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 834 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 842: A total of 19 bands was amplified with primer ISSR 842 (Figure 3.39). The largest band size was 1581 bp and the smallest band was 300 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.19.

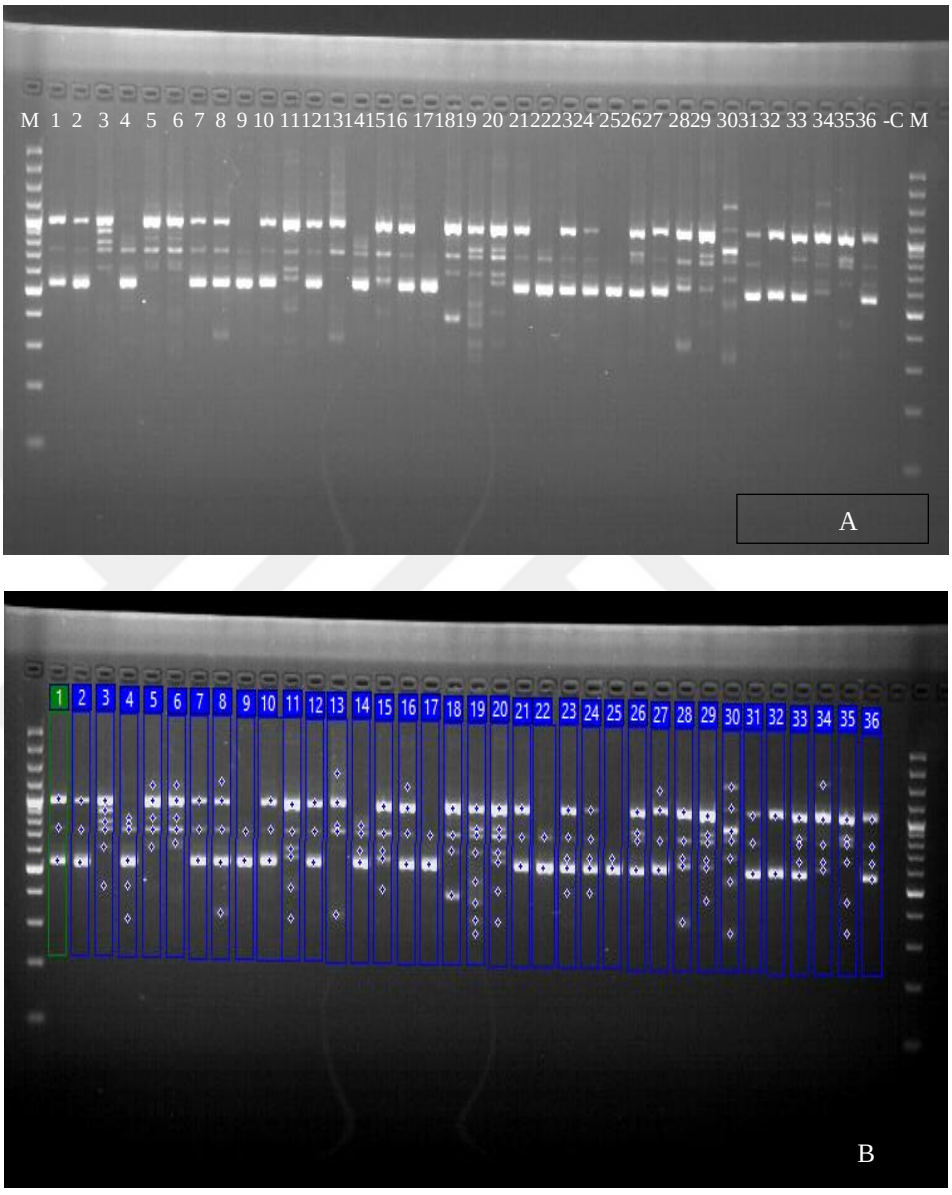


Figure 3.39. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 842 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 847: A total of 16 bands was amplified with primer ISSR 847 (Figure 3.40). The largest band size was 2397 bp and the smallest band was 443 bp, the polymorphism percentage of the primer was 94 % and PIC was calculated as 0.33.

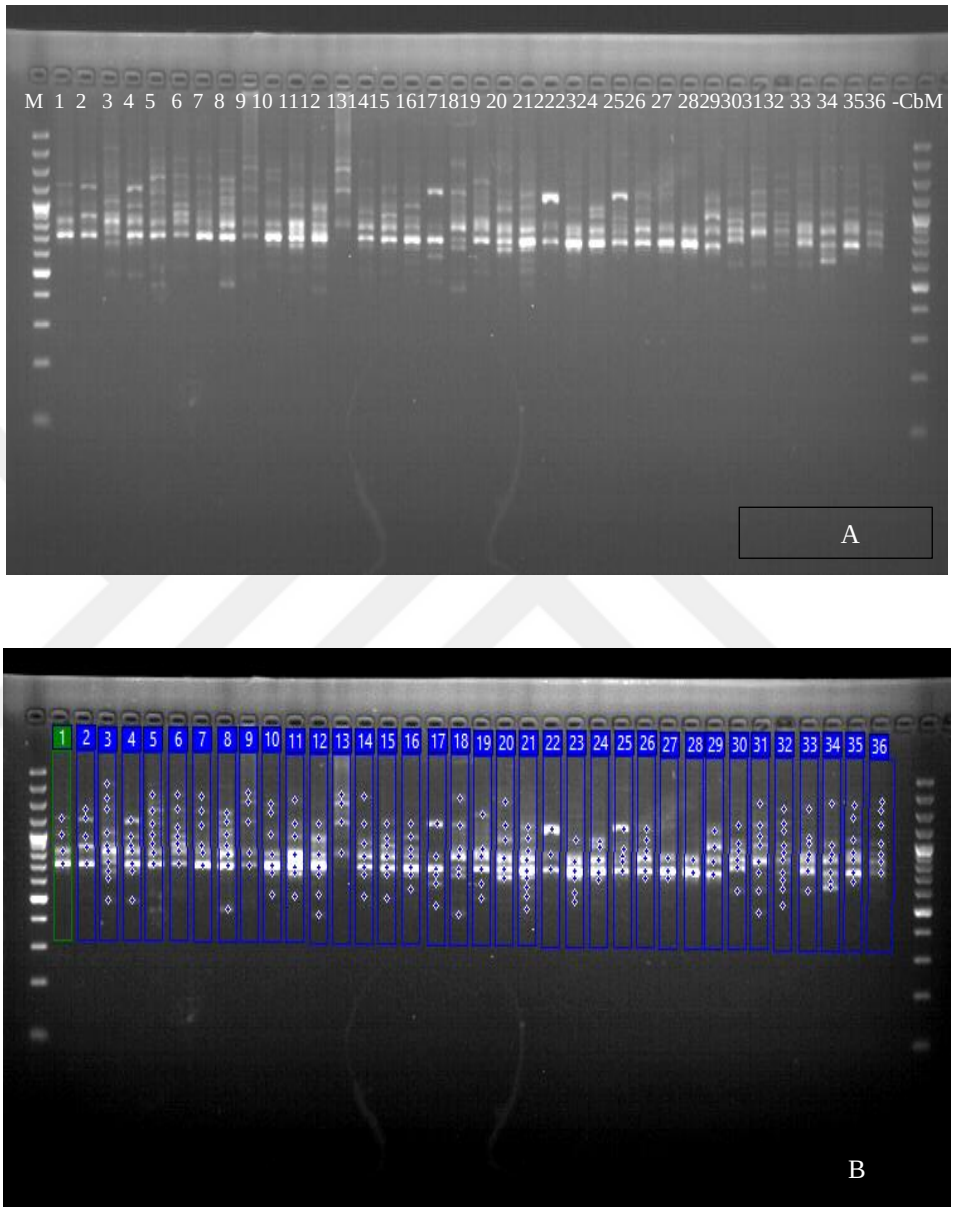


Figure 3.40. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 847 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 851: A total of 19 bands was amplified with primer ISSR 851 (Figure 3.41). The largest band size was 1920 bp and the smallest band was 408 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.38.

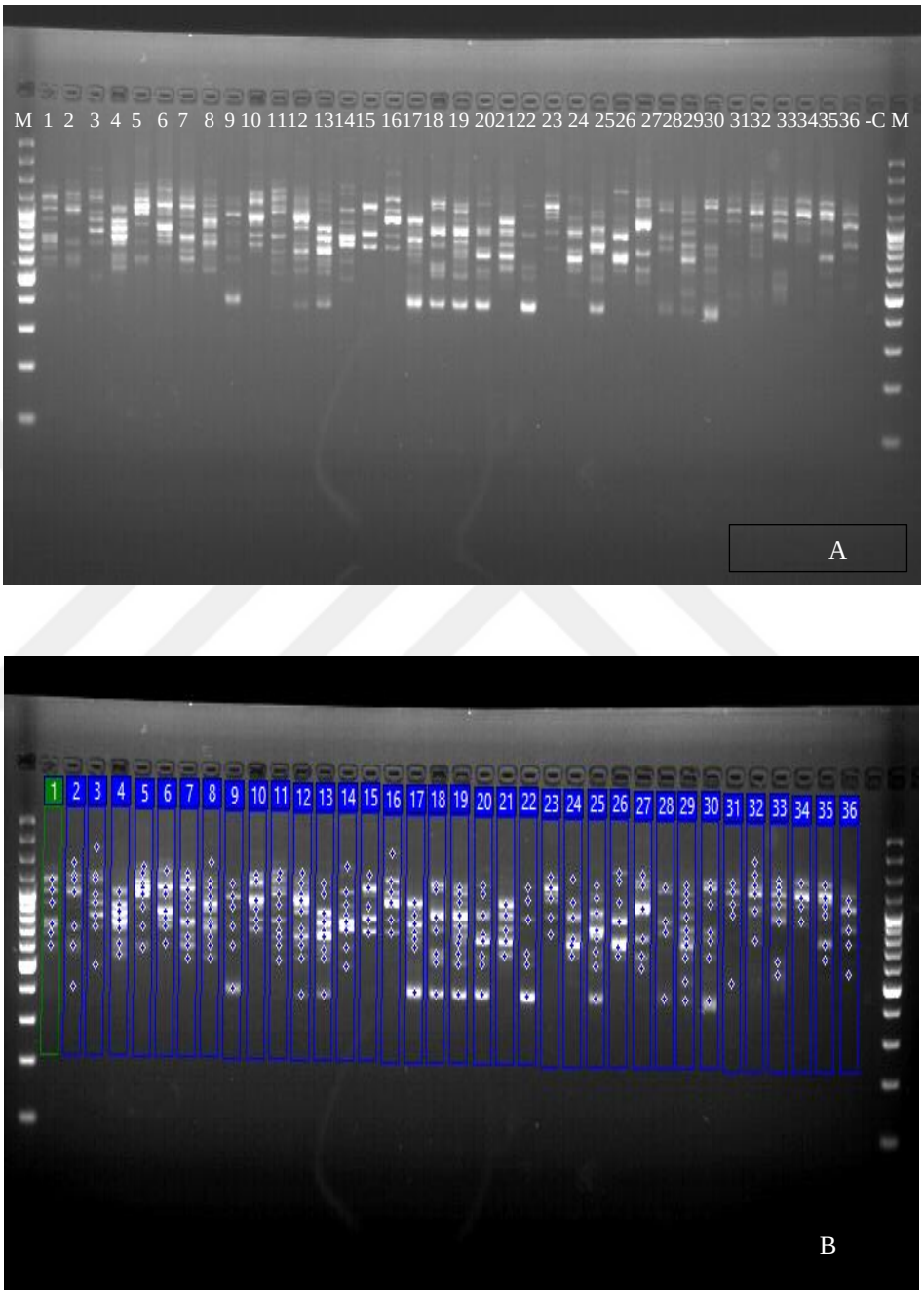


Figure 3.41. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 851 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

ISSR 859: A total of 19 bands was amplified with primer ISSR 859 (Figure 3.42). The largest band size was 3667 bp and the smallest band was 345 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.28.

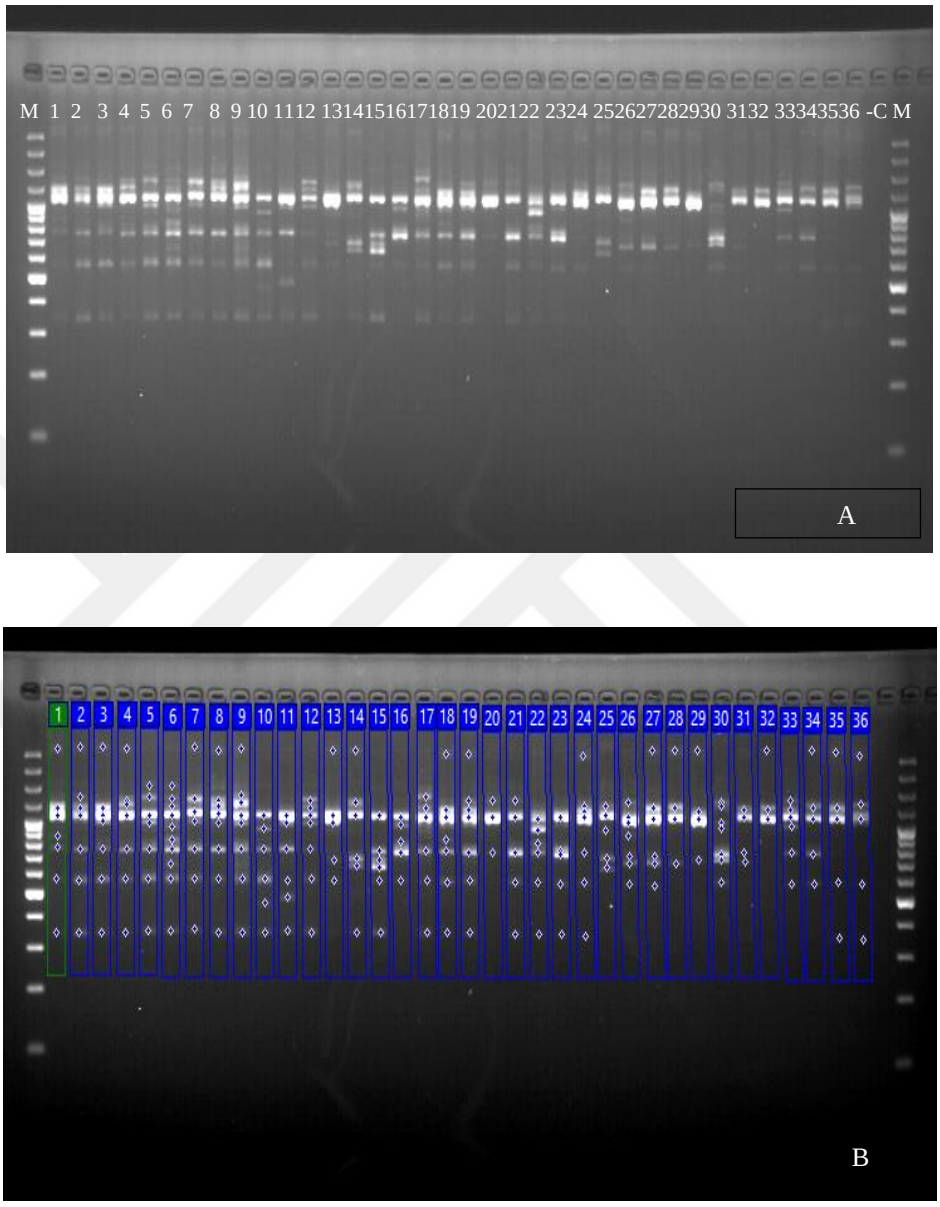


Figure 3.42. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer ISSR 859 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

3.5. SCoT Primer Optimization

In total 19 SCoT primers were tested for chickpea and almond accessions (table 3.7 and 3.9), and 18 SCoT primers for lentil accessions (Table 3.8). 10 SCoT primers were chosen due to amplifying clear and reproducible band profiles for further PCR analyses.

Table 3.8. *Tested SCoT primers for chickpea DNA accessions.*

	Primer code	Sequences (5'-3')	Original temp.	Tested temp.	Proper temp.	Results
1	SCoT 1	CAACAA**ATG**GCTACCACCA	54	50, 52, 54	52	Light bands
2	SCoT 2	CAACAA**ATG**GCTACCACCC	56	50, 52, 54	54	Light bands
3	**SCoT 3**	CAACAA**ATG**GCTACCACCG	56	52, 54, 56, 58	52	**Full set**
4	SCoT 4	CAACAA**ATG**GCTACCACCT	54	-	-	Did not work
5	SCoT 6	CAACAA**ATG**GCTACCACGC	56	50, 52, 54, 56, 58	54	Light bands
6	**SCoT 9**	CAACAA**ATG**GCTACCAGCA	54	50, 52, 54, 56, 58	54	Light bands
7	SCoT 10	CAACAA**ATG**GCTACCAGCC	56	50, 52, 54	54	Incomplete set
8	**SCoT 12**	ACGAC**ATG**GCGACCAACG	58	52, 54, 56, 58	56	**Full set**
9	**SCoT 15**	ACGAC**ATG**GCGACCGCGA	61	50, 52, 54, 56, 58	54	**Full set**
10	**SCoT 16**	ACC**ATG**GCTACCACCGAC	58	50, 52, 53, 54, 55, 56, 58	52	**Full set**
11	**SCoT 18**	ACC**ATG**GCTACCACCGCC	61	50, 52, 54, 56, 58, 60, 61	54	**Full set**
12	**SCoT 19**	ACC**ATG**GCTACCACCGGC	61	50, 52, 54, 56, 58, 60, 61	54	**Full set**
13	**SCoT 21**	ACGAC**ATG**GCGACCCACA	58	52, 53, 54, 55, 56, 58	52	**Full set**
14	SCoT 25	ACC**ATG**GCTACCACCGGG	61	50	-	Did not work
15	SCoT 24	CACC**ATG**GCTACCACCAT	58	54	-	Did not work
16	**SCoT 28**	CC**ATG**GCTACCACCGCCA	61	50, 52, 54, 56, 58	50	**Full set**
17	**SCoT 32**	CC**ATG**GCTACCACCGCAC	61	50, 52, 54, 56, 58	50	**Full set**
18	SCoT 33	CC**ATG**GCTACCACCGCAG	61	50	-	Light bands
19	SCoT 34	ACC**ATG**GCTACCACCGCA	56	50	-	Light bands

Table 3.9. *Tested SCoT primers for lentil DNA accessions.*

	Primer code	Sequences (5'-3')	Original temp.	Tested temp.	Proper temp.	Results
1	SCoT 2	CAACAA**ATG**GCTACCACCC	56	52, 54, 56, 58	52	Light bands
2	**SCoT 3**	CAACAA**ATG**GCTACCACCG	56	52, 54, 56, 58	56	**Full set**
3	SCoT 4	CAACAA**ATG**GCTACCACCT	54	50, 52, 54, 56	54	Light bands
4	SCoT 6	CAACAA**ATG**GCTACCACGC	56	52, 54, 56, 58	56	Light bands
5	SCoT 10	CAACAA**ATG**GCTACCAGCC	56	50, 52, 54, 56	-	Did not work
6	SCoT 12	ACGAC**ATG**GCGACCAACG	58	52, 54, 56, 58	-	Did not work
7	**SCoT 15**	ACGAC**ATG**GCGACCGCGA	61	50, 52, 54, 56, 58	54	**Full set**
8	**SCoT 16**	ACC**ATG**GCTACCACCGAC	58	52.53, 54, 55, 56, 58	52	**Full set**
9	**SCoT 17**	ACC**ATG**GCTACCACCGAG	58	50, 52, 54, 56, 58	54	**Full set**
10	**SCoT 18**	ACC**ATG**GCTACCACCGCC	61	52, 54,56, 58, 60, 61	56	**Full set**
11	**SCoT 19**	ACC**ATG**GCTACCACCGGC	61	52, 54,56, 58, 60, 61	52	**Full set**
12	SCoT 20	ACC**ATG**GCTACCACCGCG	61	50, 52, 54, 56, 58, 60	58	Light Bands
13	**SCoT 21**	ACGAC**ATG**GCGACCCACA	58	52, 53, 54, 55, 56, 58	52	**Full set**
14	SCoT 26	ACC**ATG**GCTACCACCGTC	58	50, 52, 54, 56, 58	56	Light bands
15	**SCoT 28**	CC**ATG**GCTACCACCGCCA	61	50, 52, 54, 56, 58	56	**Full set**
16	**SCoT 32**	CC**ATG**GCTACCACCGCAC	61	50, 52, 54, 56, 58	50	**Full set**
17	**SCoT 34**	ACC**ATG**GCTACCACCGCA	56	50, 52, 54, 56, 58	52	**Full set**
18	SCoT 36	GCAACAA**ATG**GCTACCACC	56	50, 52, 54, 56	-	Did not work

Table 3.10. *Tested SCoT primers for almond DNA accessions.*

	Primer code	Sequences (5'-3')	Original temp.	Tested temp.	Proper temp.	Results
1	SCoT 1	CAACAA**ATG**GCTACCACCA	54	50, 52, 54	-	Did not work
2	SCoT 2	CAACAA**ATG**GCTACCACCC	56	52, 54, 56, 58	-	Did not work
3	SCoT 4	CAACAA**ATG**GCTACCACCT	54	50, 52, 54, 56	54	Light Bands
4	SCoT 6	CAACAA**ATG**GCTACCACGC	56	52, 54, 56, 58	54	Light Bands
5	SCoT 9	CAACAA**ATG**GCTACCAGCA	54	52, 54, 56, 58	54	Light Bands
6	SCoT 10	CAACAA**ATG**GCTACCAGCC	56	50, 52, 54, 56	-	Did not work
7	**SCoT 12**	ACGAC**ATG**GCGACCAACG	58	52, 54, 56, 58	56	**Full Set**
8	**SCoT 15**	ACGAC**ATG**GCGACCGCGA	61	50, 52, 54, 56, 58	52	**Full Set**
9	**SCoT 16**	ACC**ATG**GCTACCACCGAC	58	52.53, 54, 55, 56, 58	52	**Full Set**
10	**SCoT 17**	ACC**ATG**GCTACCACCGAG	58	50, 52, 54, 56, 58	52	**Full Set**
11	**SCoT 18**	ACC**ATG**GCTACCACCGCC	61	52, 54,56, 58, 60, 61	54	**Full Set**
12	**SCoT 19**	ACC**ATG**GCTACCACCGGC	61	52, 54,56, 58, 60, 61	52	**Full Set**
13	SCoT 20	ACC**ATG**GCTACCACCGCG	61	50, 52, 54, 56, 58, 60	58	Light Bands
14	**SCoT 21**	ACGAC**ATG**GCGACCCACA	58	52, 53, 54, 55, 56, 58	53	**Full Set**
15	SCoT 26	ACC**ATG**GCTACCACCGTC	58	50, 52, 54, 56, 58	-	Did not work
16	**SCoT 28**	CC**ATG**GCTACCACCGCCA	61	50, 52, 54, 56, 58	54	**Full Set**
17	**SCoT 32**	CC**ATG**GCTACCACCGCAC	61	50, 52, 54, 56, 58	52	**Full Set**
18	**SCoT 34**	ACC**ATG**GCTACCACCGCA	56	50, 52, 54, 56, 58	50	**Full Set**
19	SCoT 36	GCAACA**ATG**GCTACCACC	56	50, 52, 54, 56	-	Did not work

3.6. Results of SCoT-PCR Analysis

3.6.1. SCoT-PCR analysis of chickpea accessions

PCR results of 10 SCoT primers used to amplify DNA from 18 chickpea accessions were visualized under the UV light and digitally photographed with gel documentation system (Figures 3.43-3.52).

SCoT 1: A total of 13 bands was amplified with primer SCoT 1 (Figure 3.43). The largest band size was 2650 bp and the smallest was 588 bp, the polymorphism percentage of the primer was 92 % and the PIC was calculated as 0.35.

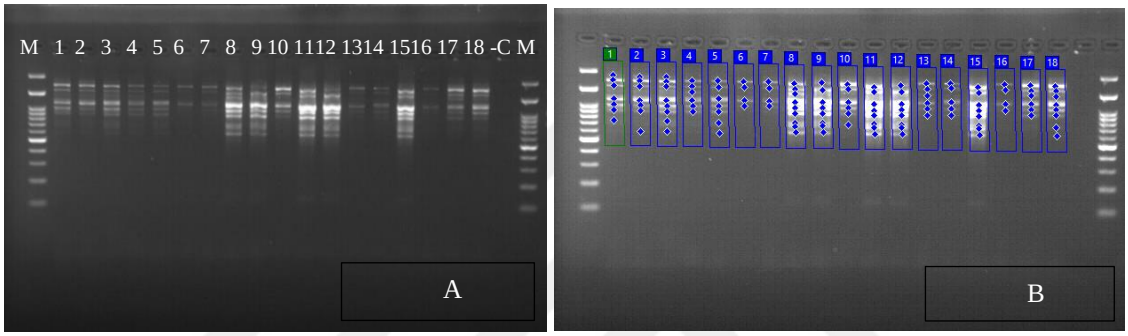


Figure 3.43. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 1 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 3: A total of 8 bands were amplified with primer SCoT 3 (Figure 3.44). The largest band size was 2935 bp and the smallest band was 800 bp, the polymorphism percentage of the primer was 50 % and the PIC was calculated as 0.19.

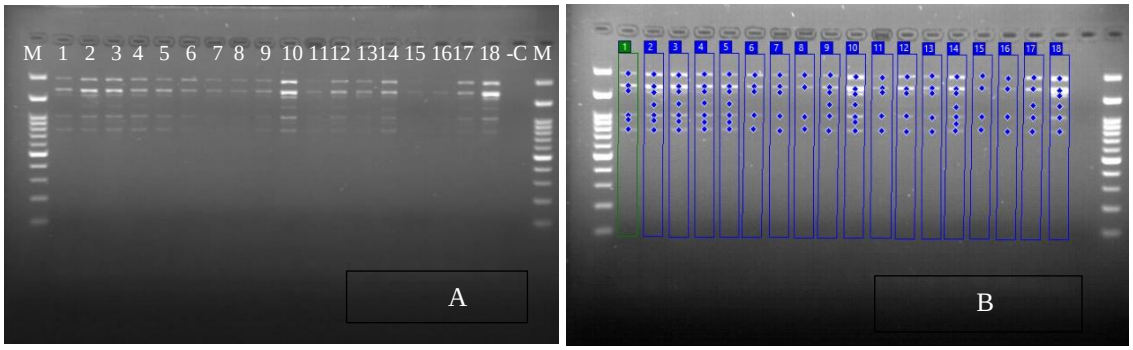


Figure 3.44. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 3 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 12: A total of 20 bands were amplified with primer SCoT 12 (Figure 3.45). The largest band size was 2729 bp and the smallest band was 185 bp, the polymorphism percentage of the primer was 80 % and the PIC was calculated as 0.26.

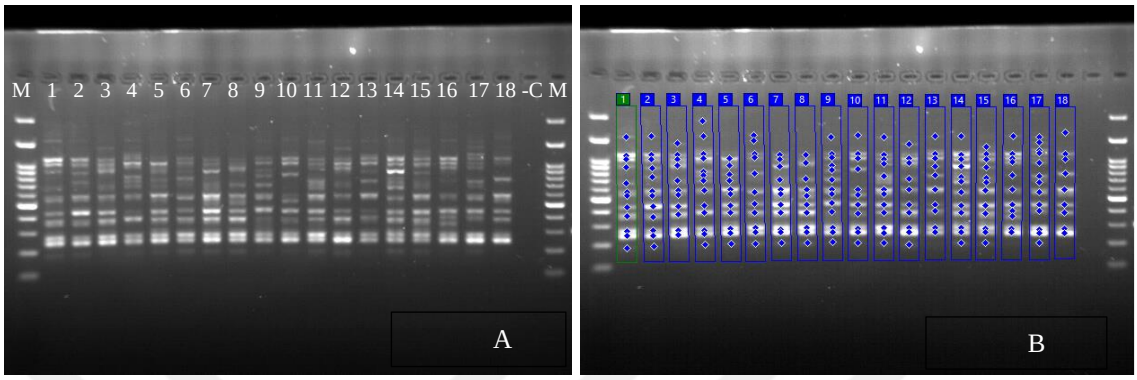


Figure 3.45. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 12 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 15: A total of 21 bands were amplified with primer SCoT 15 (Figure 3.46). The largest band size was 2911 bp and the smallest band was 225 bp, the polymorphism percentage of the primer was 86 % and the PIC was calculated as 0.31.

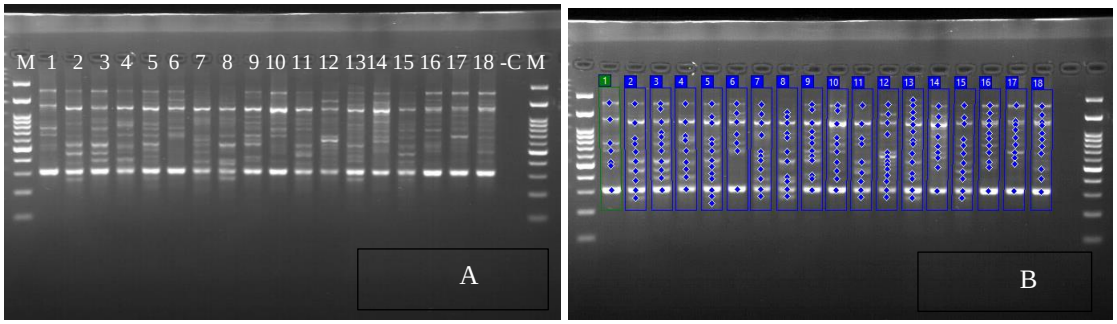


Figure 3.46. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 15 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 16: A total of 20 bands were amplified with primer SCoT 16 (Figure 3.47). The largest band size was 3404 bp and the smallest band was 288 bp, the polymorphism percentage of the primer was 90 % and the PIC was calculated as 0.30.

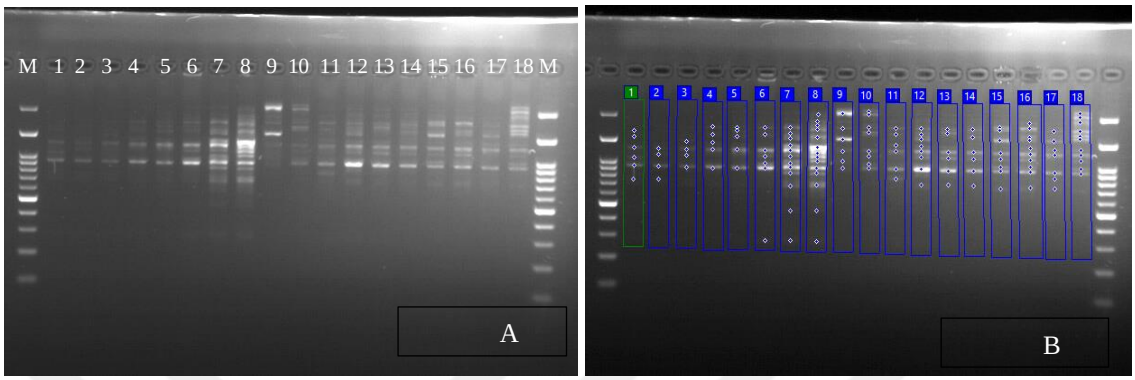


Figure 3.47. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 16 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

SCoT 18: A total of 13 bands were amplified with primer SCoT 18 (Figure 3.48). The largest band size was 2927 bp and the smallest band was 227 bp, the polymorphism percentage of the primer was 54 % and the PIC was calculated as 0.13.

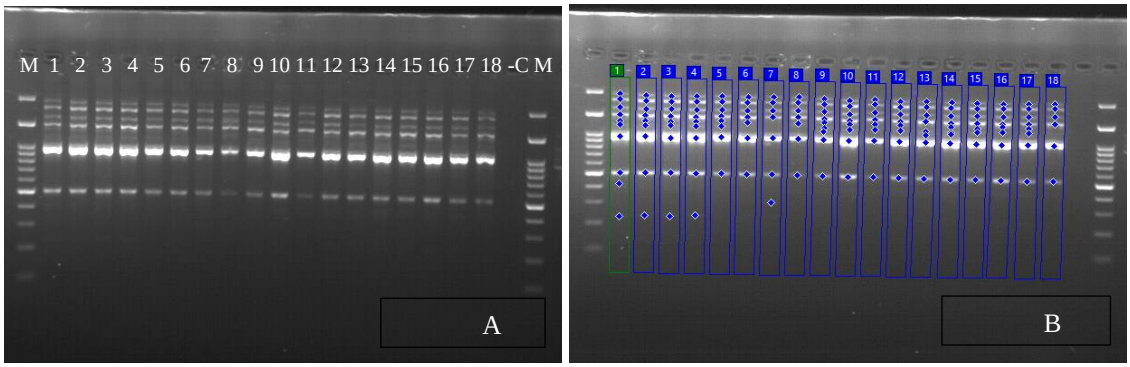


Figure 3.48. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 18 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 19: A total of 17 bands were amplified with primer SCoT 19 (Figure 3.49). The largest band size was 3825 bp and the smallest band was 688 bp, the polymorphism percentage of the primer was 88 % and the PIC was calculated as 0.31.

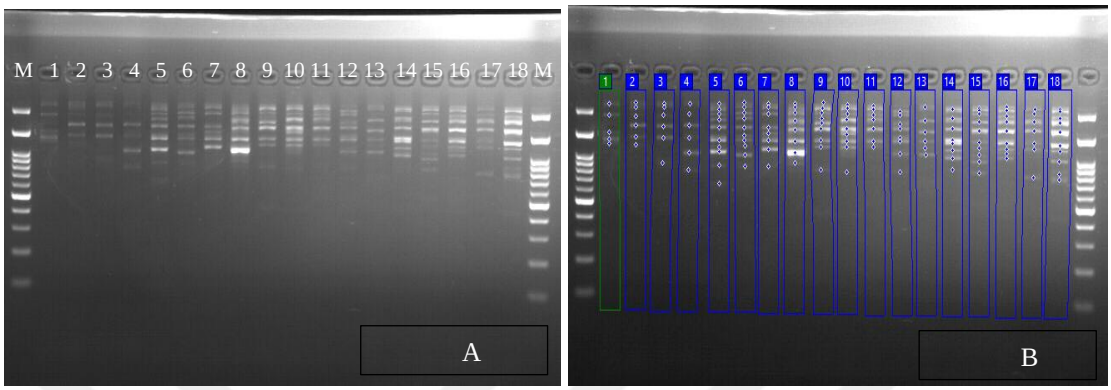


Figure 3.49. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 19 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

SCoT 21: A total of 14 bands were amplified with primer SCoT 21 (Figure 3.50). The largest band size was 1733 bp and the smallest band was 230 bp, the polymorphism percentage of the primer was 71 % and PIC was calculated as 0.21.

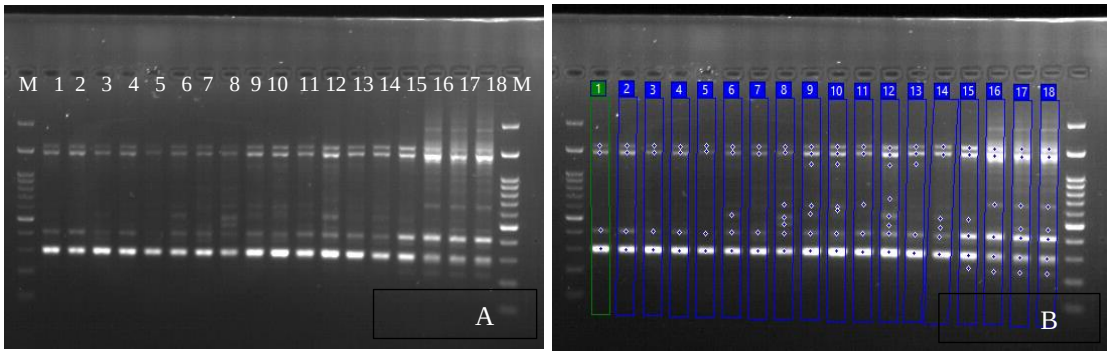


Figure 3.50. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 21 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

SCoT 28: A total of 15 bands were amplified with primer SCoT 28 (Figure 3.51). The largest band size was 3048 bp and the smallest band was 295 bp, the polymorphism percentage of the primer was 60 % and the PIC was calculated as 0.24.

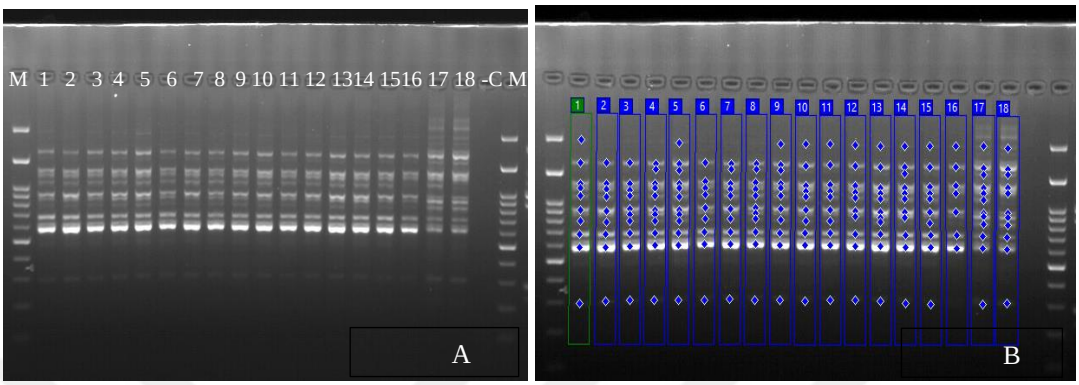


Figure 3.51. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 28 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 32: A total of 25 bands were amplified with primer SCoT 32 (Figure 3.52). The largest band size was 3281 bp and the smallest band was 240 bp, the polymorphism percentage of the primer was 80 % and the PIC was calculated as 0.26.

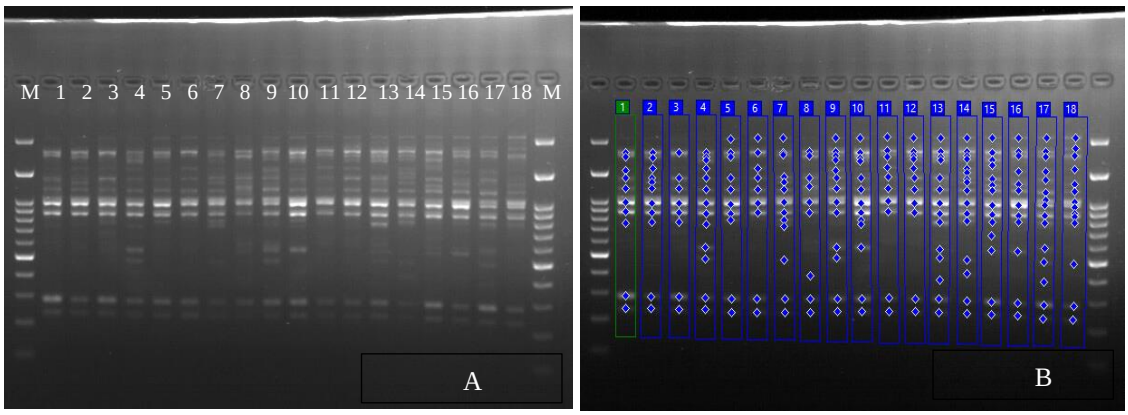


Figure 3.52. *PCR results showing band patterns obtained from 18 chickpea accessions (1-18) with primer SCoT 32 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker.*

3.6.2. SCoT-PCR analysis for the lentil accessions

10 SCoT primers were used to amplify 35 lentil accessions and their agarose gels were visualized under the UV light and digitally photographed (Figures 3.53- 3.62).

SCoT 3: A total of 20 bands were amplified with primer SCoT 3 (Figure 3.53). The largest band size was 2588 bp and the smallest band was 461 bp, the polymorphism percentage of the primer was 100 % and PIC was calculated as 0.37.

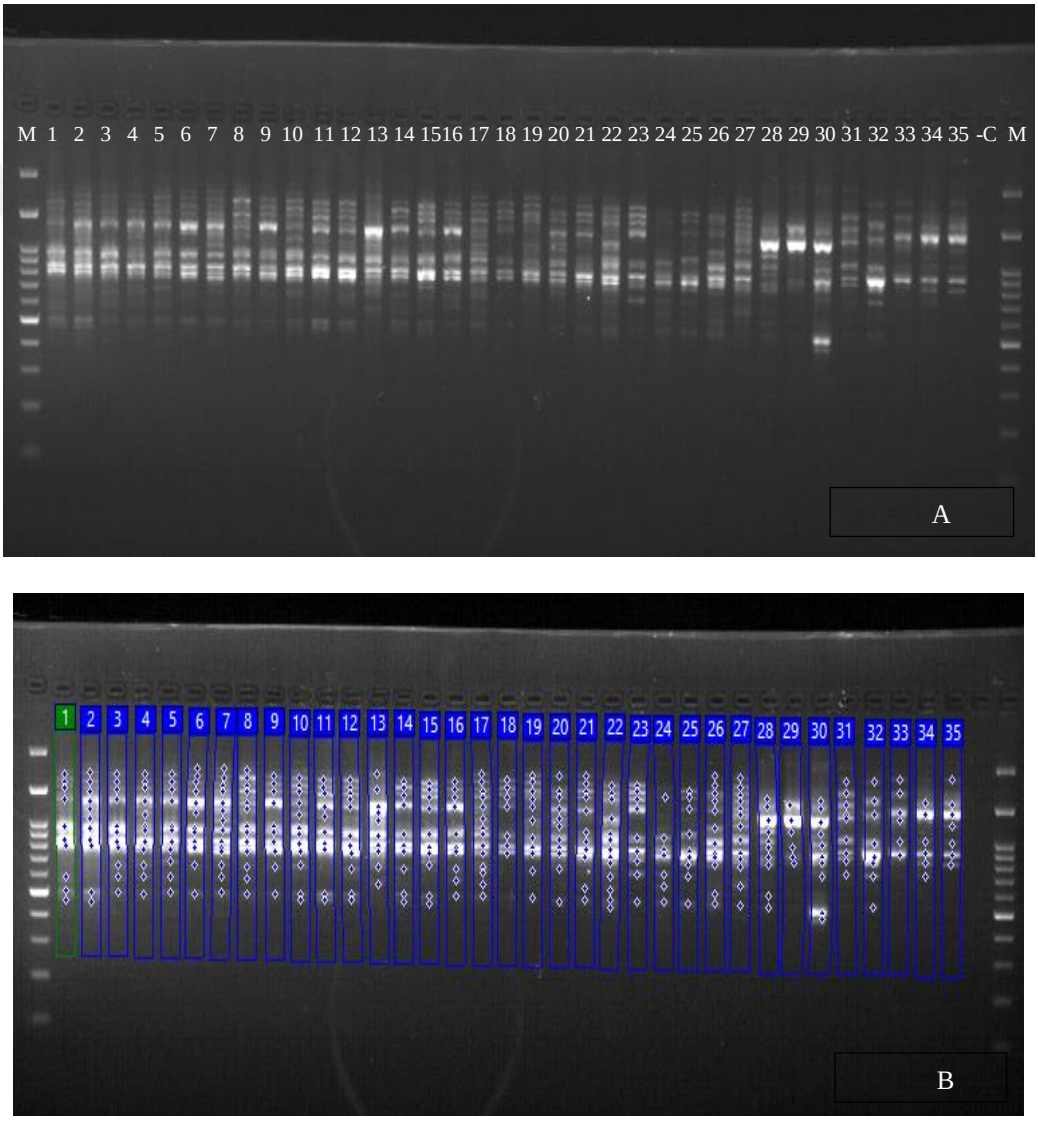


Figure 3.53. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 3 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 15: A total of 16 bands were amplified with primer SCoT 15 (Figure 3.54). The largest band size was 2244 bp and the smallest band was 310 bp, the polymorphism percentage of the primer was 88 % and PIC was calculated as 0.3.

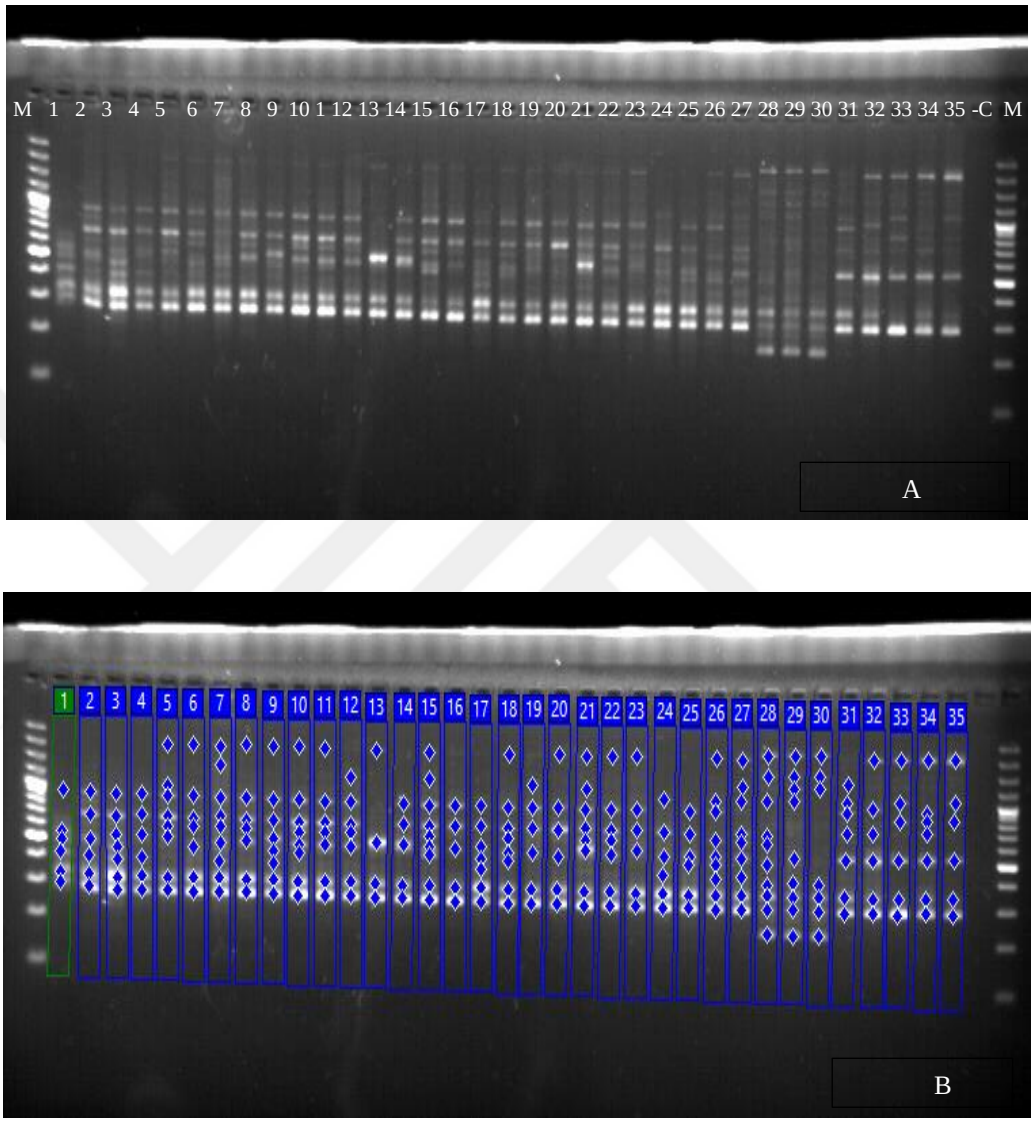


Figure 3.54. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 15 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 16: A total of 19 bands were amplified with primer SCoT 16 (Figure 3.55). The largest band size was 3720 bp and the smallest band was 675 bp, the polymorphism percentage of the primer was 84 % and PIC was calculated as 0.25.

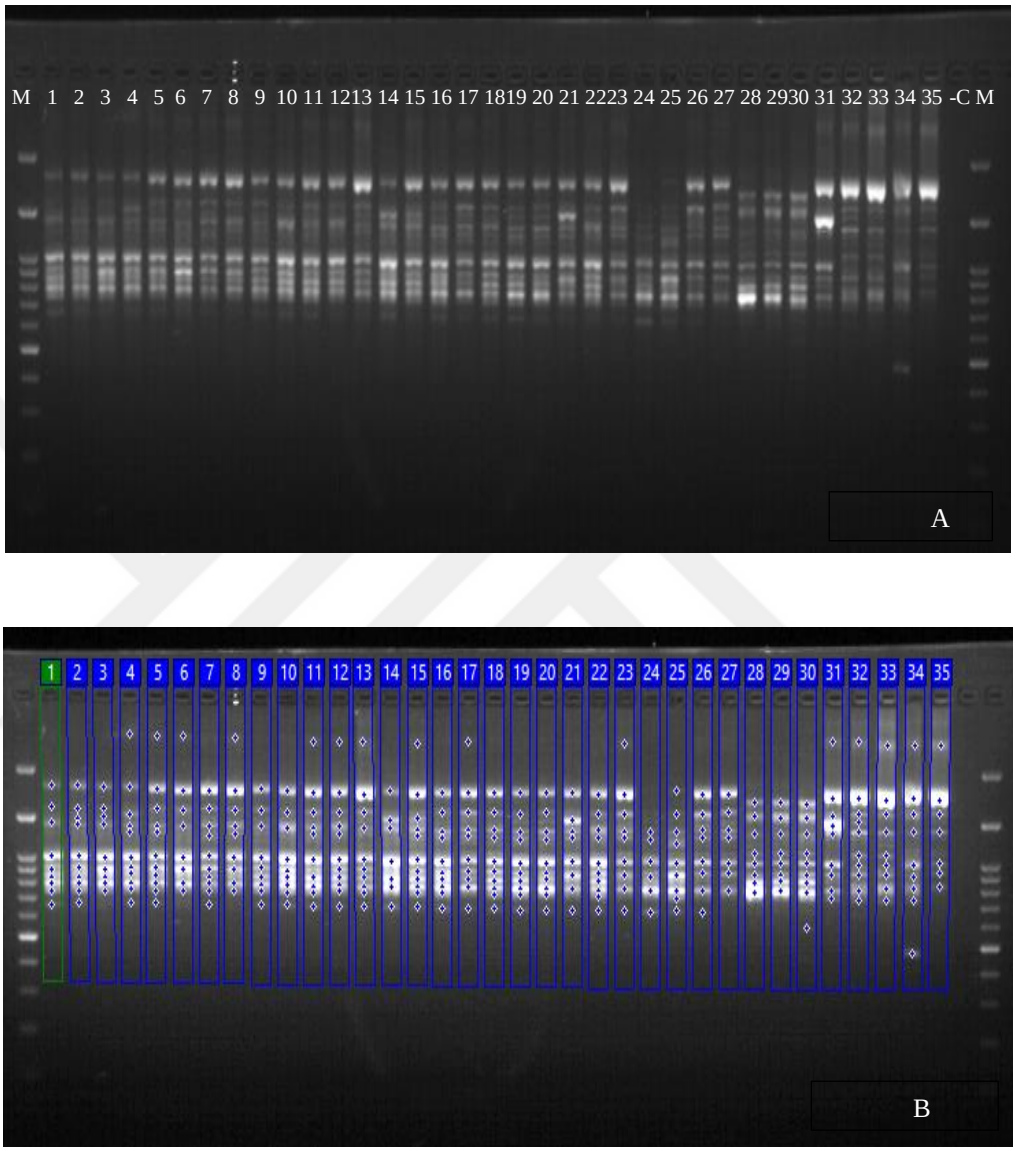


Figure 3.55. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 16 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 17: A total of 19 bands were amplified with primer SCoT 17 (Figure 3.56). The largest band size was 1620 bp and the smallest band was 247 bp, the polymorphism percentage of the primer was 84 % and PIC was calculated as 0.2.

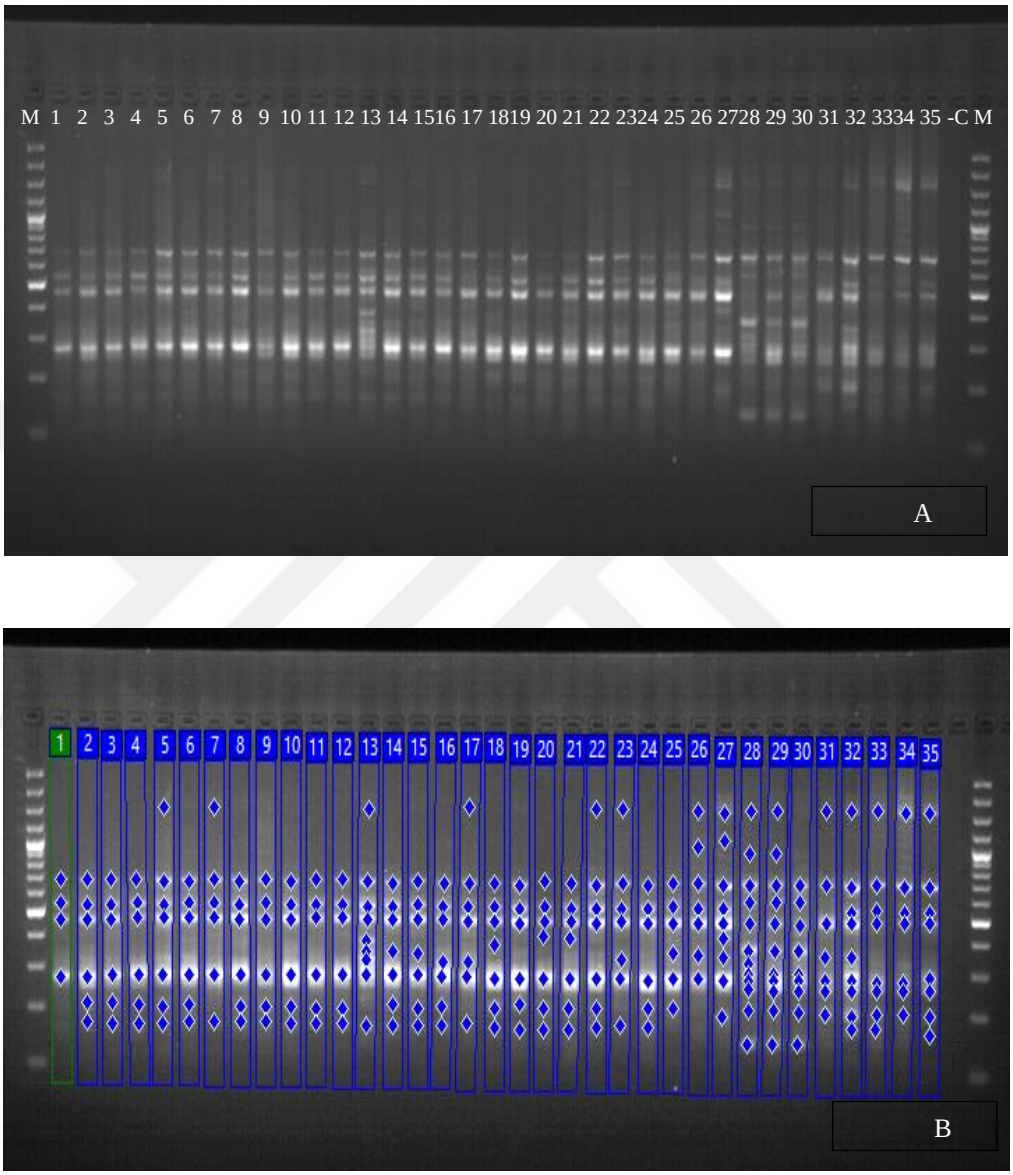


Figure 3.56. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 17 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 18: A total of 26 bands were amplified with primer SCoT 18 (Figure 3.57). The largest band size was 2454 bp and the smallest band was 355 bp, the polymorphism percentage of the primer was 96 % and PIC was calculated as 0.34.

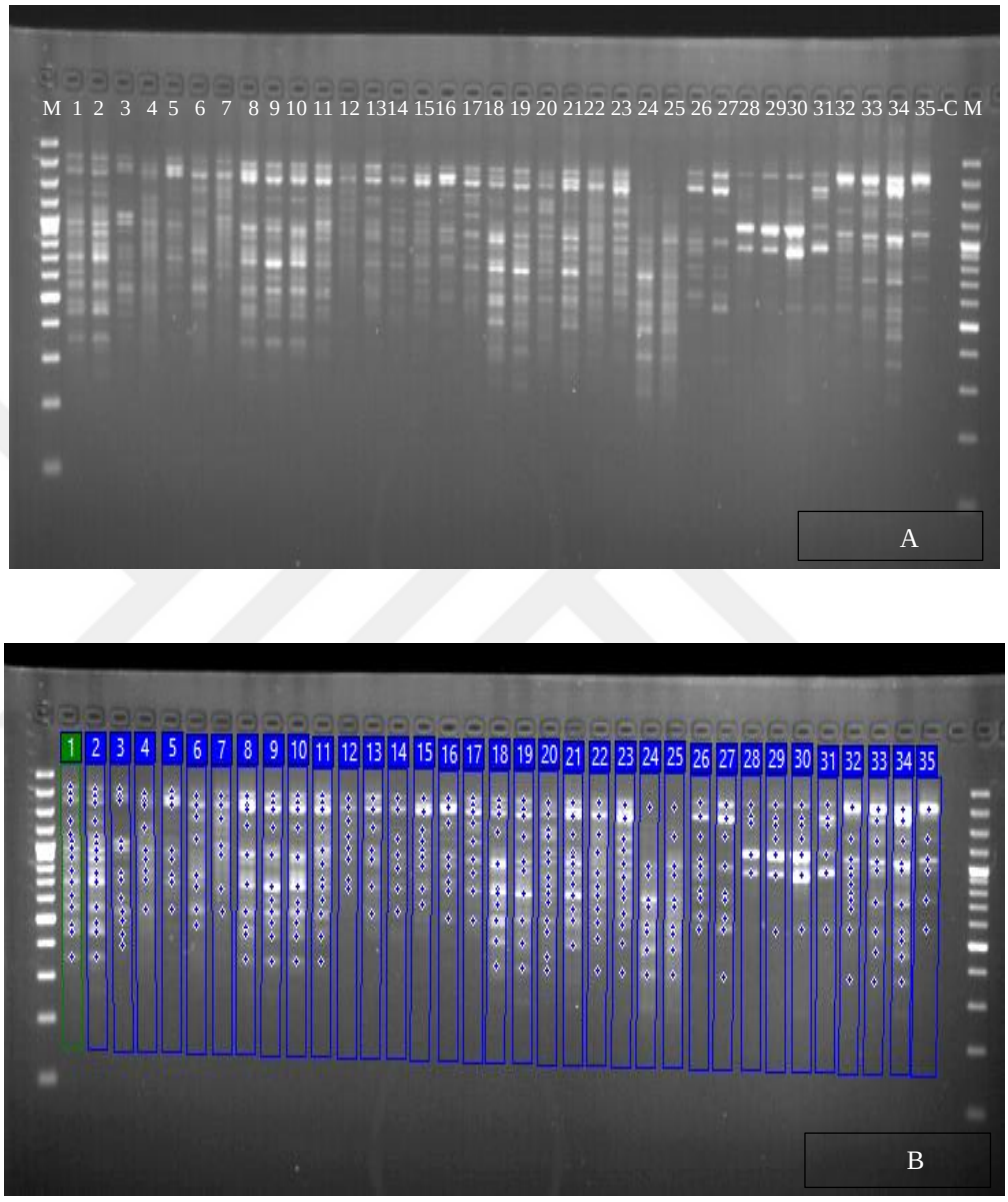


Figure 3.57. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 18 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 19: A total of 16 bands were amplified with primer SCoT 19 (Figure 3.58). The largest band size was 2689 bp and the smallest band was 625 bp, the polymorphism percentage of the primer was 94 % and PIC was calculated as 0.2.

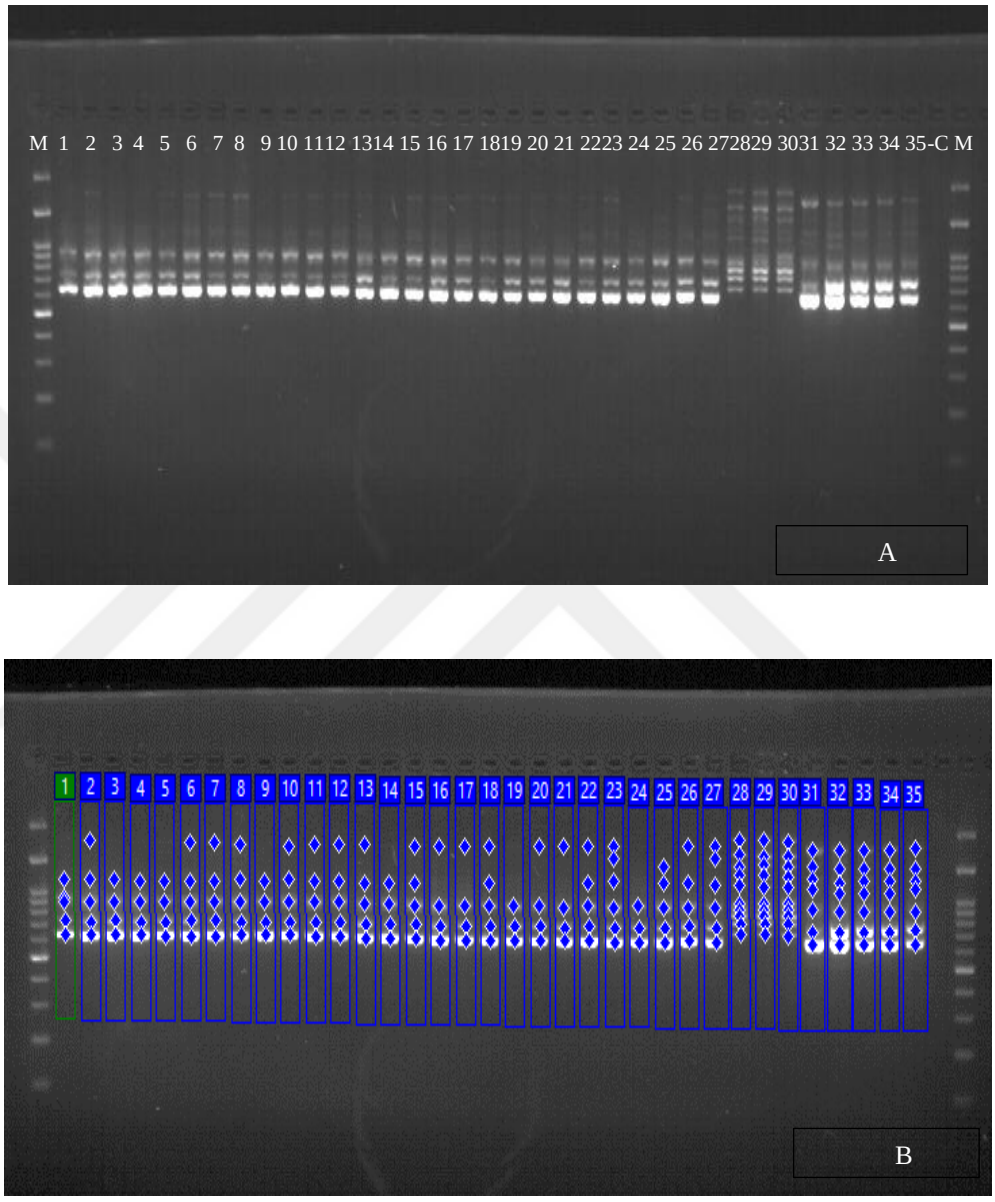


Figure 3.58. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 19 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 21: A total of 13 bands were amplified with primer SCoT 21 (Figure 3.59). The largest band size was 2254 bp and the smallest band was 370 bp, the polymorphism percentage of the primer was 85 % and PIC was calculated as 0.18.

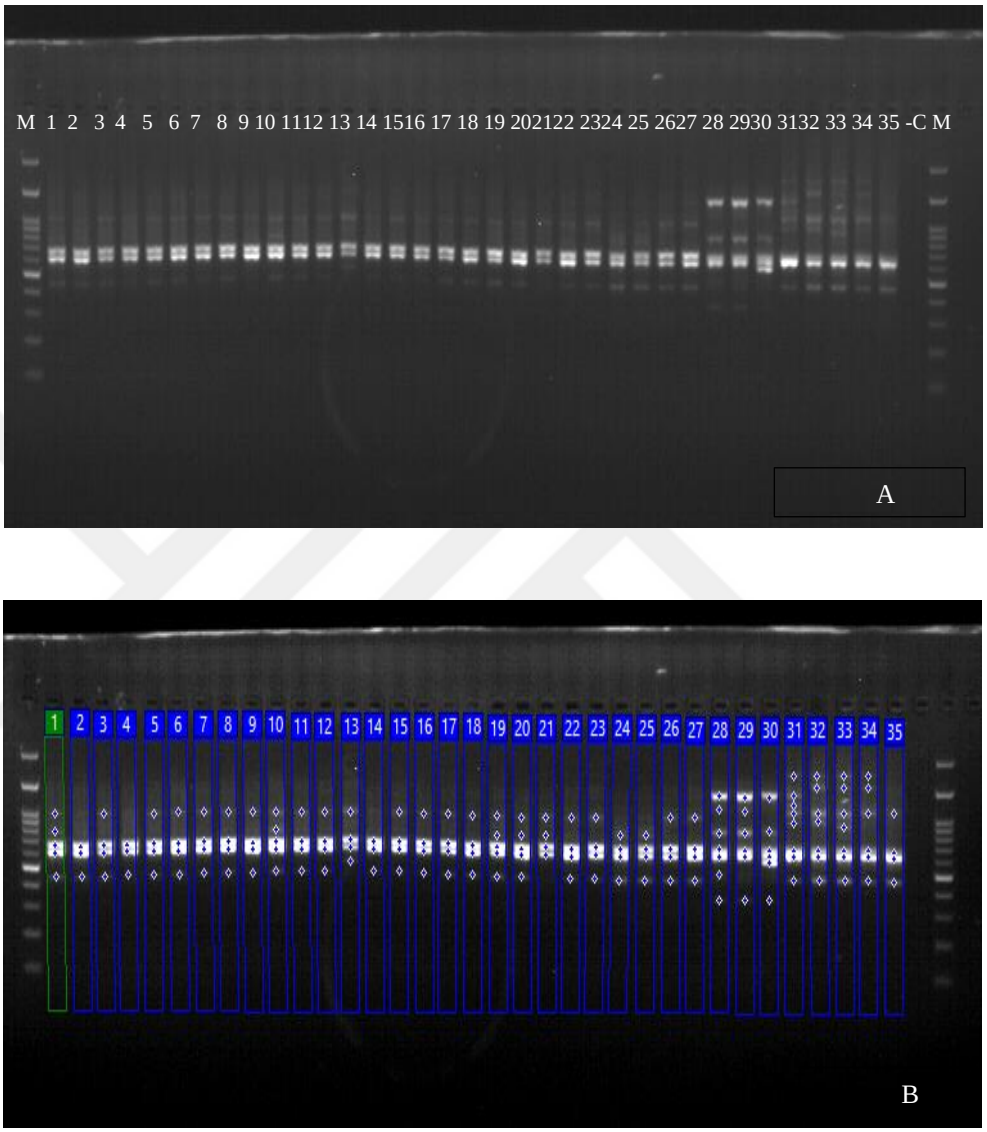


Figure 3.59. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 21 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 28: A total of 14 bands were amplified with primer SCoT 28 (Figure 3.60). The largest band size was 2200 bp and the smallest band was 265 bp, the polymorphism percentage of the primer was 86 % and PIC was calculated as 0.3.

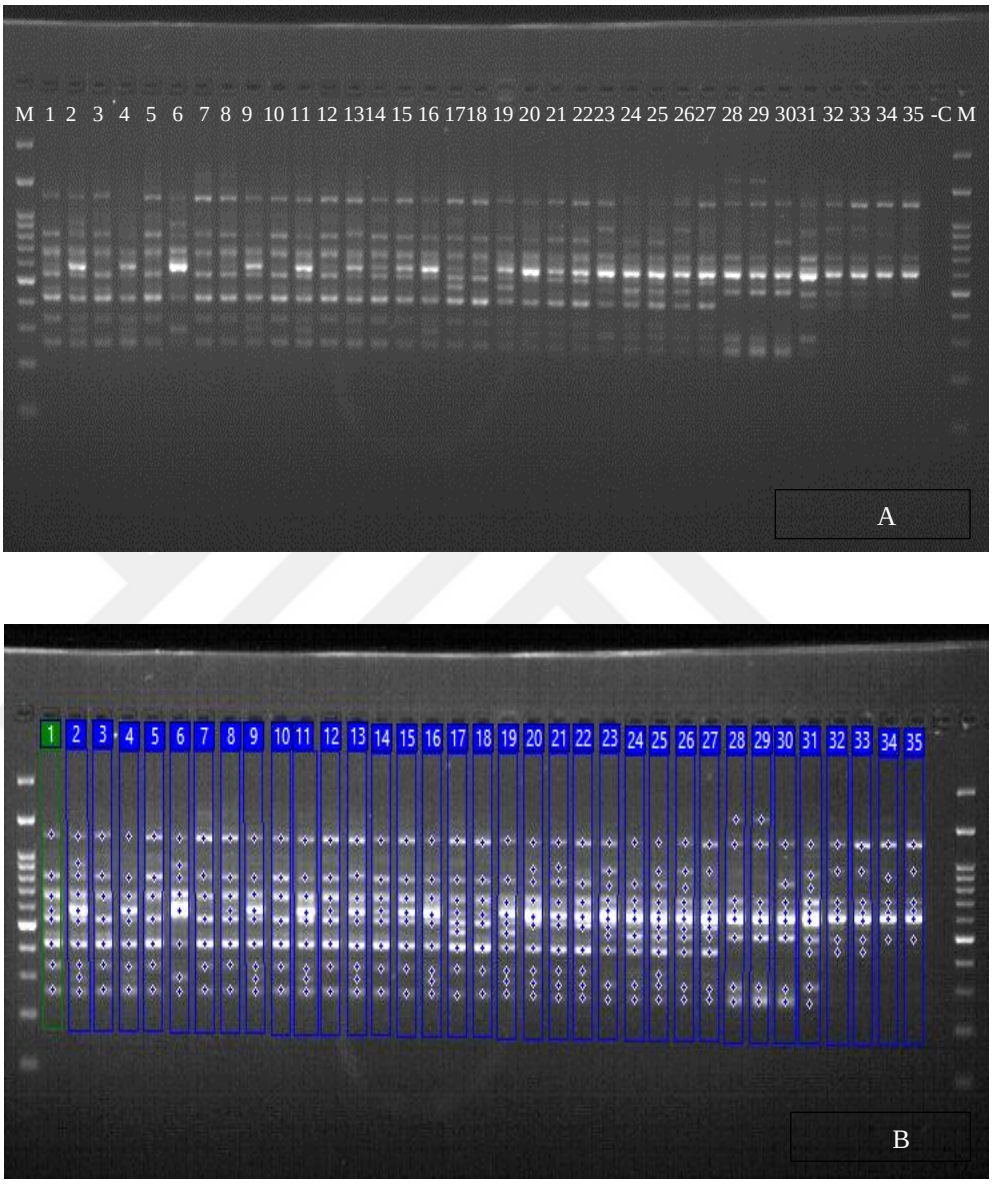


Figure 3.60. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 28 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 32: A total of 15 bands were amplified with primer SCoT 32 (Figure 3.61). The largest band size was 2537 bp and the smallest band was 279 bp, the polymorphism percentage of the primer was 80 % and PIC was calculated as 0.26.

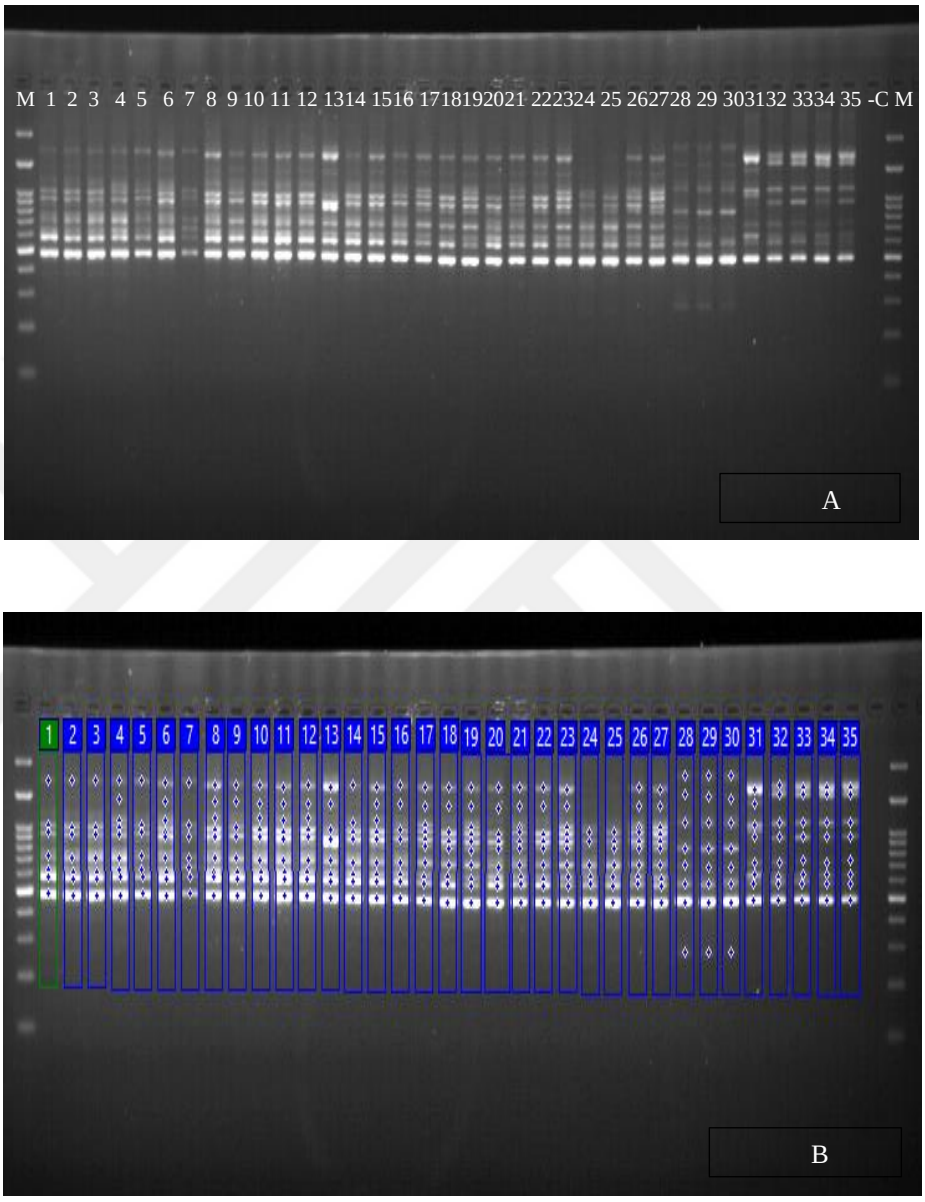


Figure 3.61. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 32 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 34: A total of 16 bands were amplified with primer SCoT 34 (Figure 3.62). The largest band size was 3500 bp and the smallest band was 330 bp, the polymorphism percentage of the primer was 88 % and PIC was calculated as 0.28.

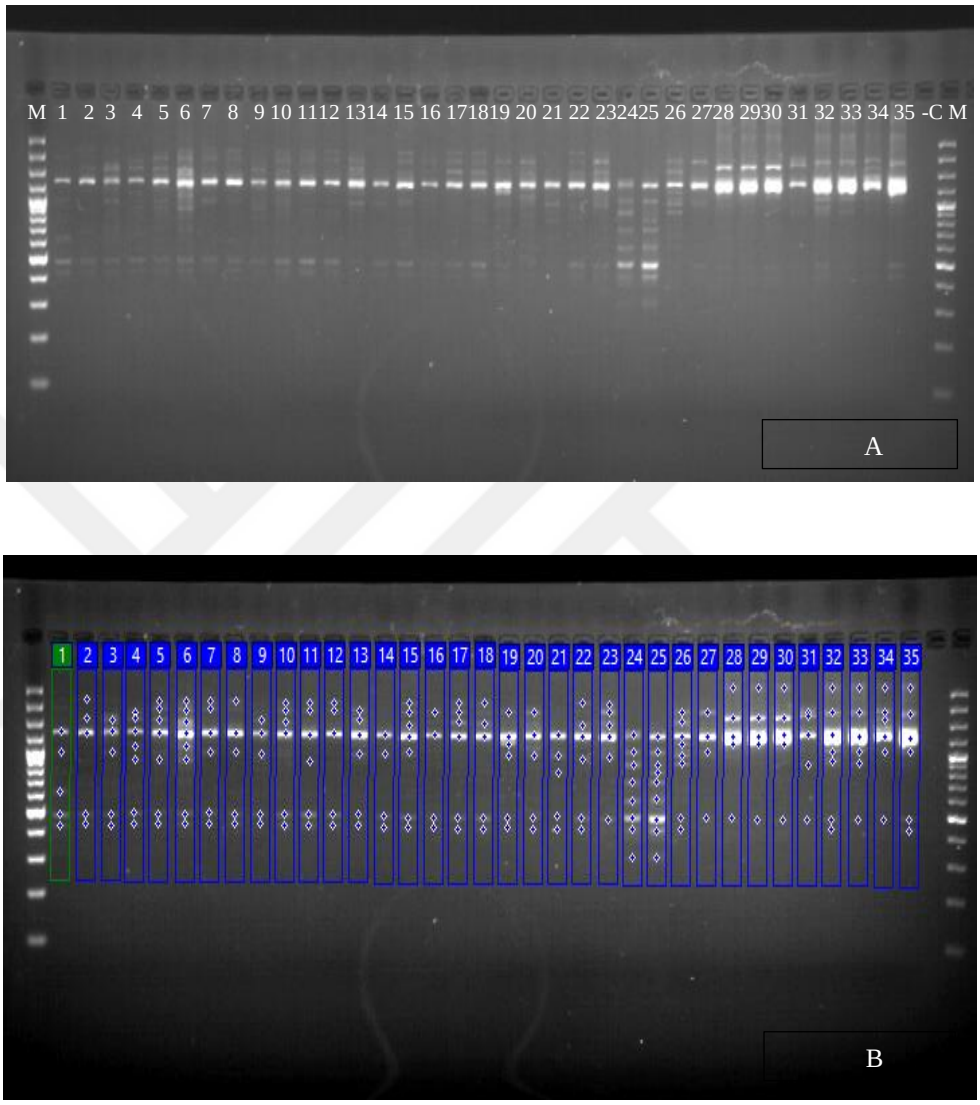


Figure 3.62. *PCR results showing band patterns obtained from 35 lentil accessions (1-35) with primer SCoT 34 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

3.6.3. SCoT-PCR analysis for the almond accessions

A total of 10 SCoT primers were used to amplify 36 almond accessions (24, cultivated; 12, wild). PCR products in agarose gels were visualized under the UV light and digitally photographed (Figures 3.63- 3.72).

SCoT 12: A total of 23 bands were amplified with primer SCoT 12 (Figure 3.63). The largest band size was 2813 bp and the smallest band was 313 bp, the polymorphism percentage of the primer was 91 % and PIC was calculated as 0.3.

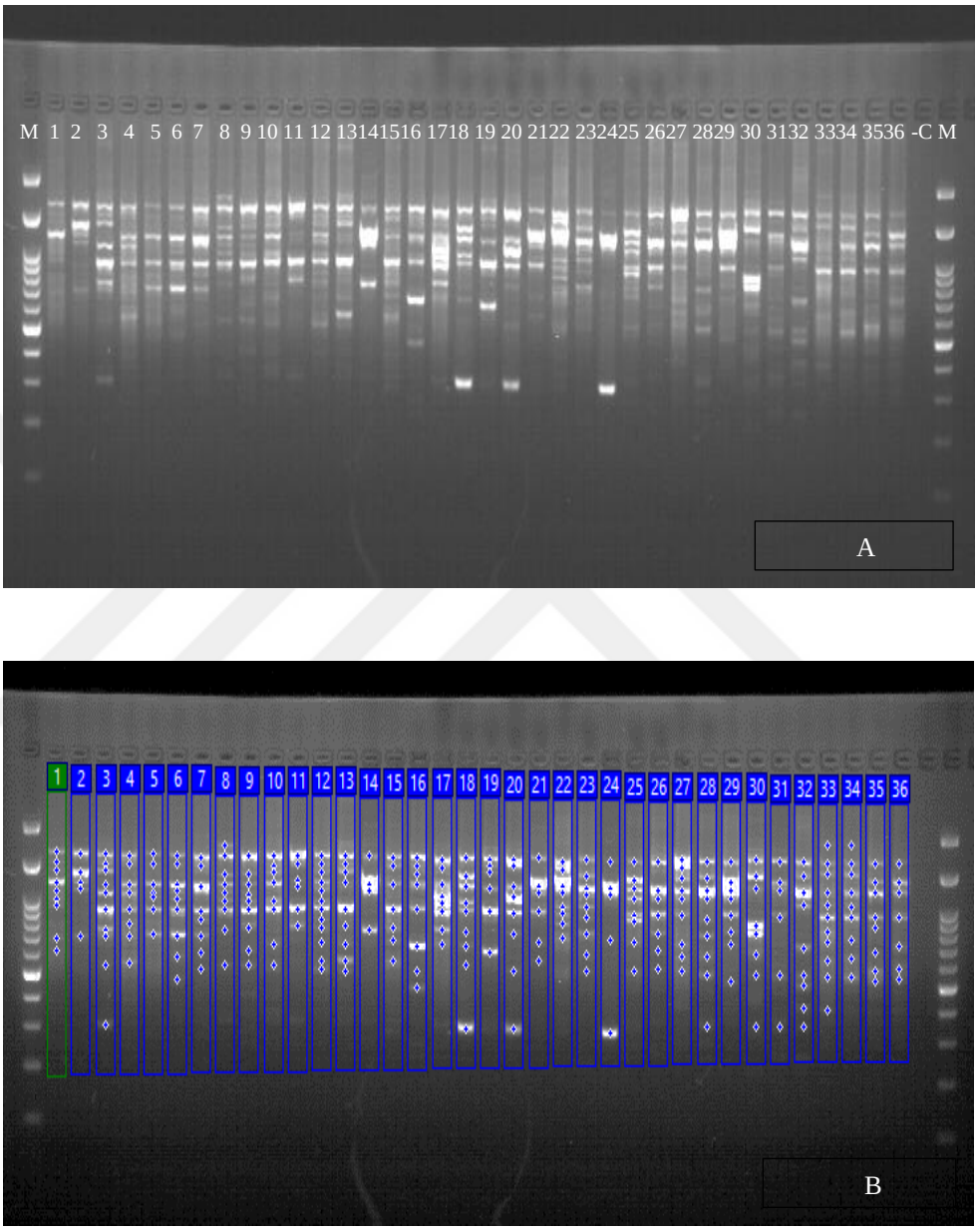


Figure 3.63. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 12 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 15: A total of 22 bands were amplified with primer SCoT 15 (Figure 3.64). The largest band size was 2630 bp and the smallest band was 282 bp, the polymorphism percentage of the primer was 86 % and PIC was calculated as 0.24.

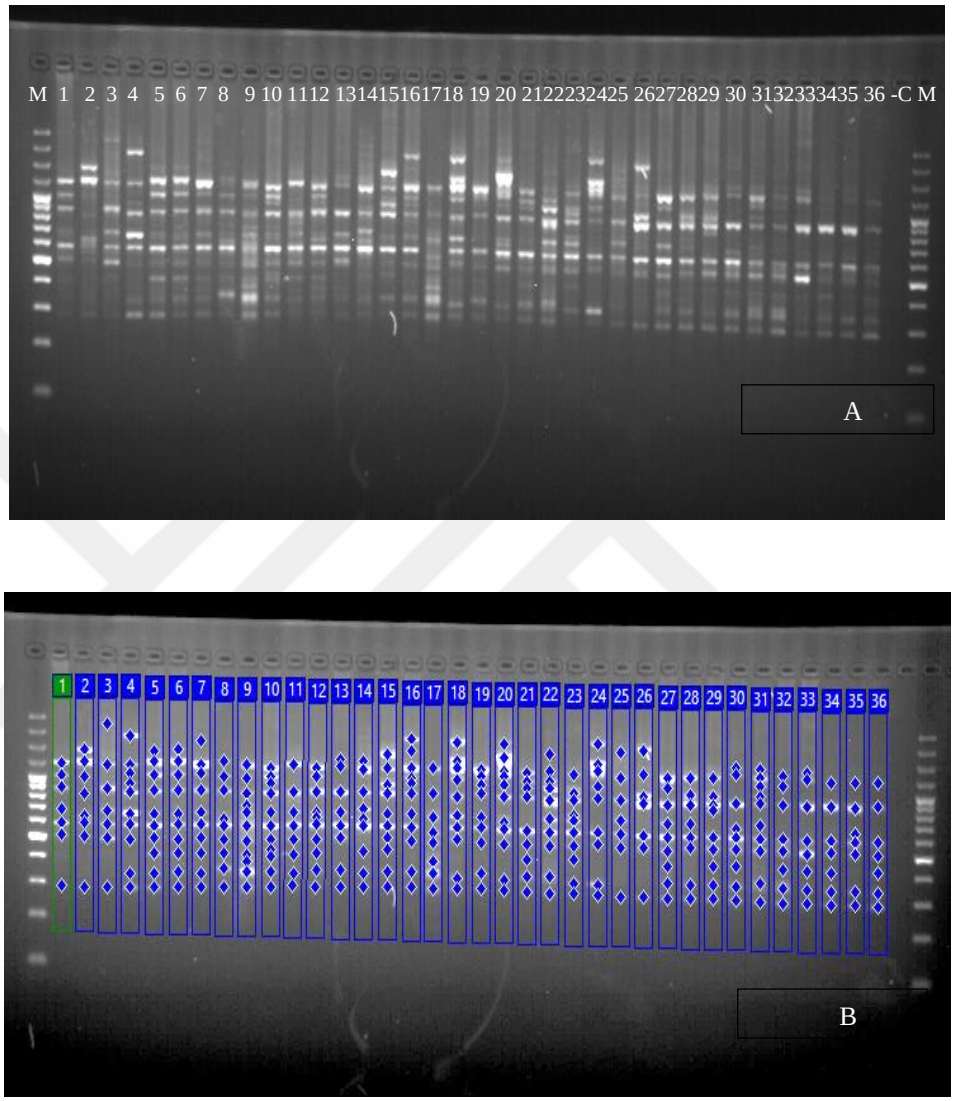


Figure 3.64. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 15 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 16: A total of 14 bands were amplified with primer SCoT 16 (Figure 3.65). The largest band size was 3681 bp and the smallest band was 687 bp, the polymorphism percentage of the primer was 93 % and PIC was calculated as 0.27.

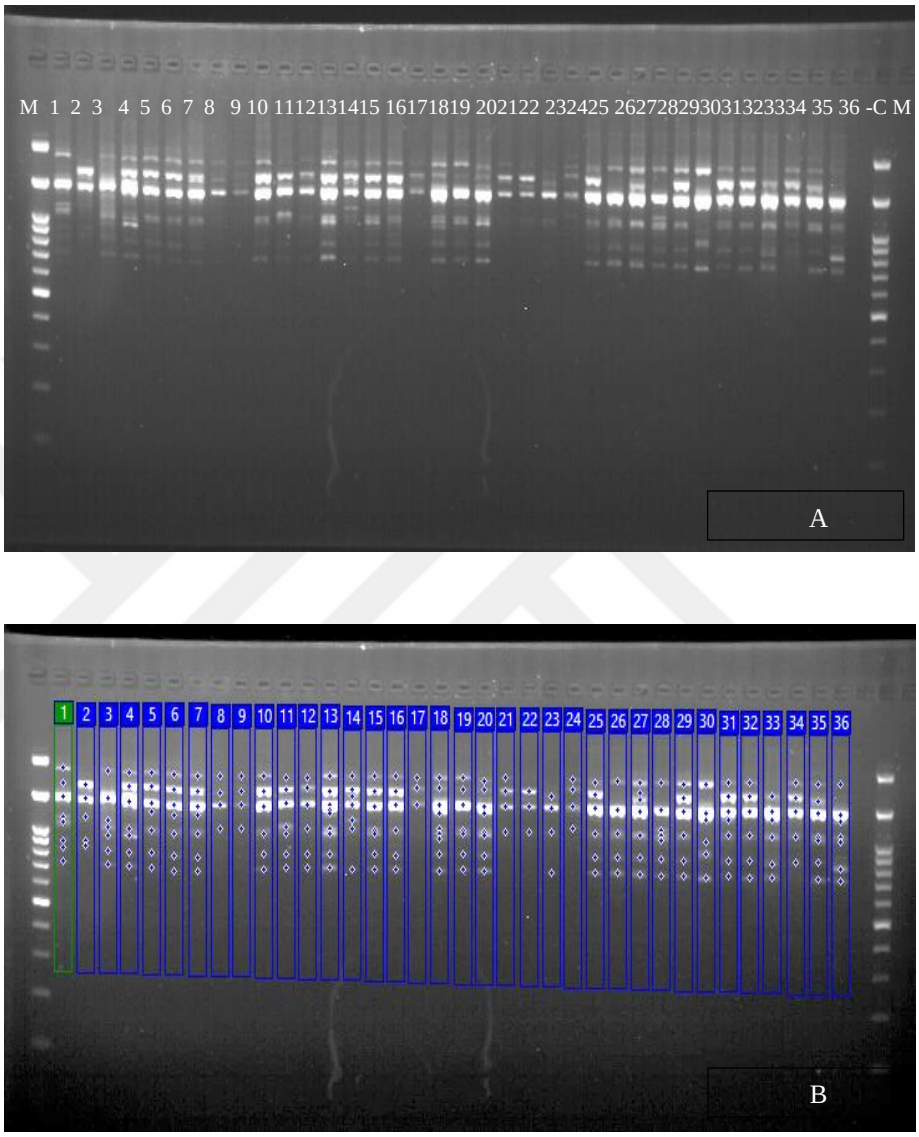


Figure 3.65. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 16 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 17: A total of 18 bands were amplified with primer SCoT 17 (Figure 3.66). The largest band size was 2548 bp and the smallest band was 242 bp, the polymorphism percentage of the primer was 94 % and PIC was calculated as 0.22.

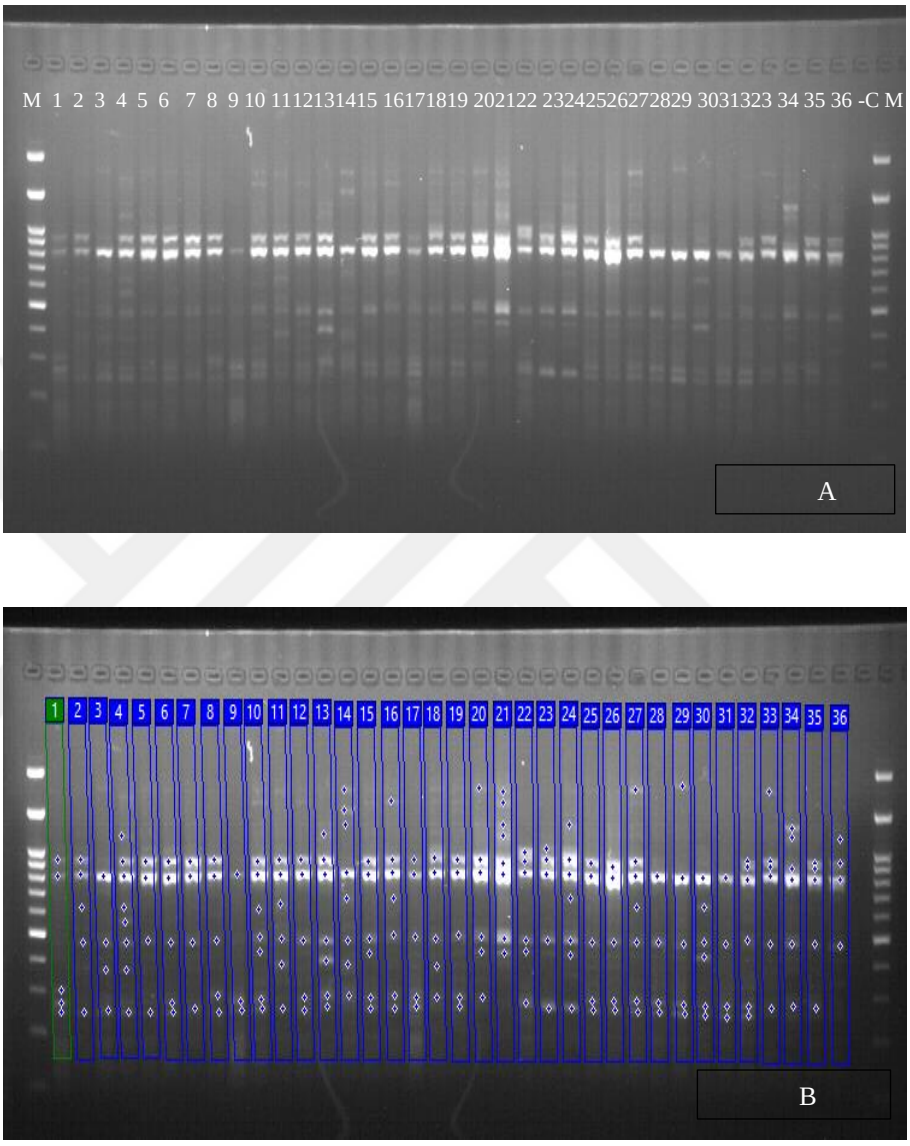


Figure 3.66. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 17 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 18: A total of 22 bands were amplified with primer SCoT 18 (Figure 3.67). The largest band size was 2823 bp and the smallest band was 323 bp, the polymorphism percentage of the primer was 86 % and PIC was calculated as 0.25.

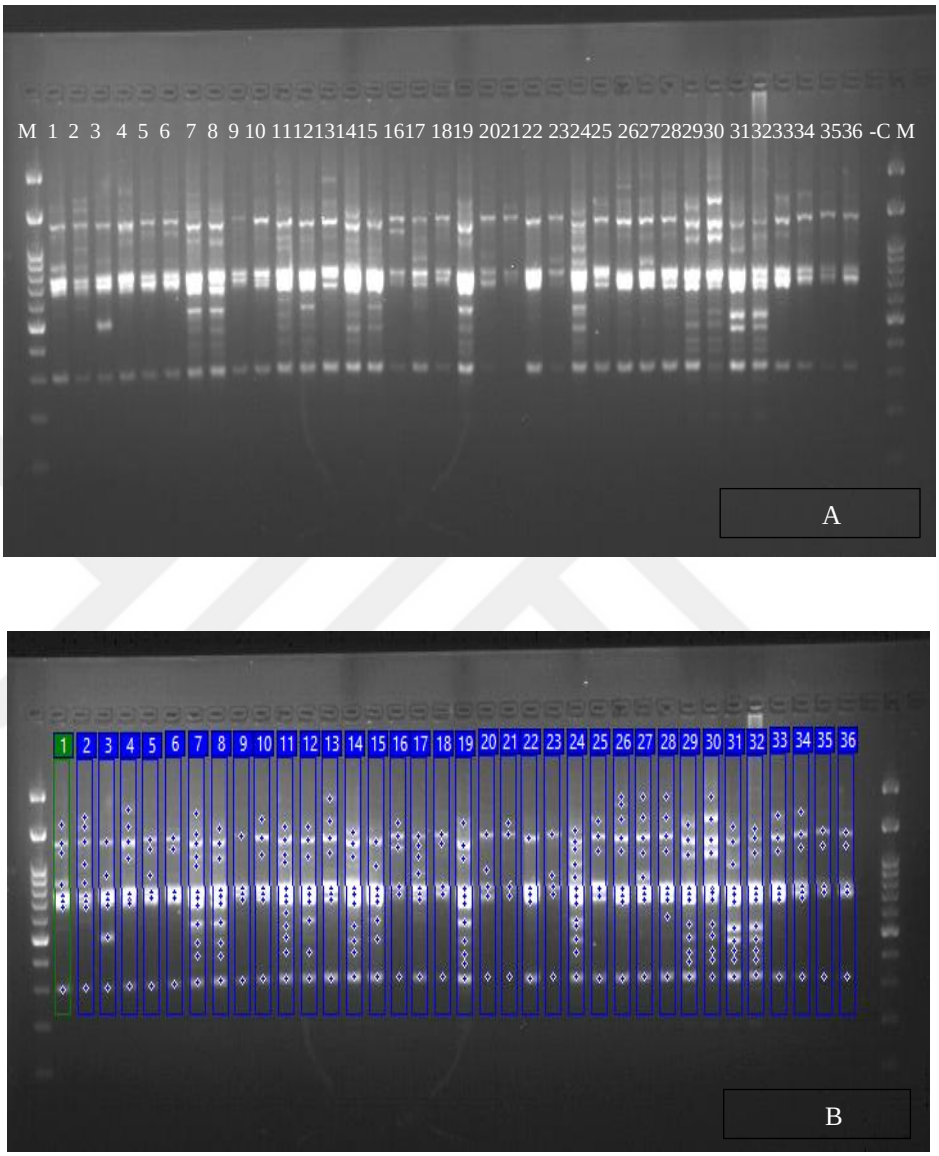


Figure 3.67. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 18 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 19: A total of 23 bands were amplified with primer SCoT 19 (Figure 3.68). The largest band size was 3000 bp and the smallest band was 482 bp, the polymorphism percentage of the primer was 91 % and PIC was calculated as 0.28.

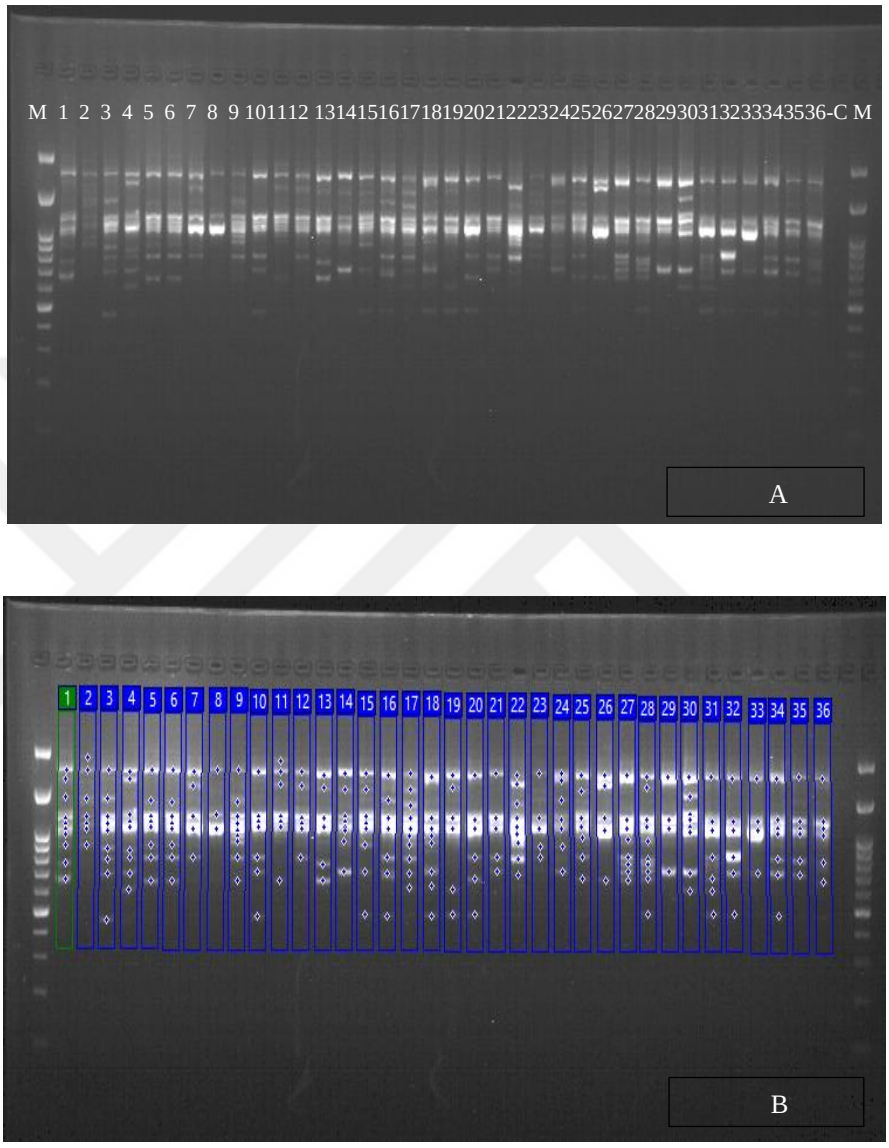


Figure 3.68. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 19 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 21: A total of 19 bands were amplified with primer SCoT 21 (Figure 3.69). The largest band size was 2791 bp and the smallest band was 240 bp, the polymorphism percentage of the primer was 84 % and PIC was calculated as 0.22.

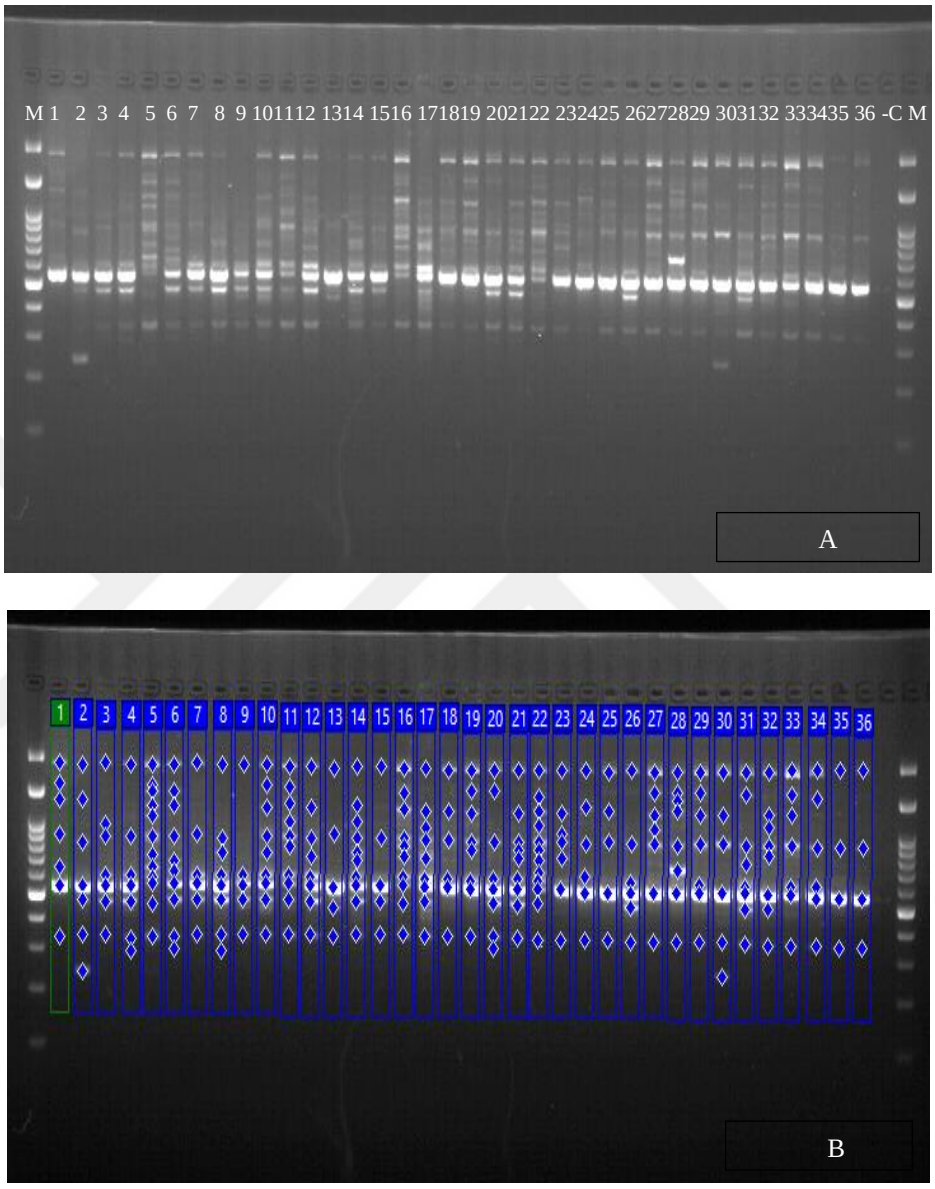


Figure 3.69. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 21 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 28: A total of 13 bands were amplified with primer SCoT 28 (Figure 3.70). The largest band size was 2974 bp and the smallest band was 452 bp, the polymorphism percentage of the primer was 85 % and PIC was calculated as 0.19.

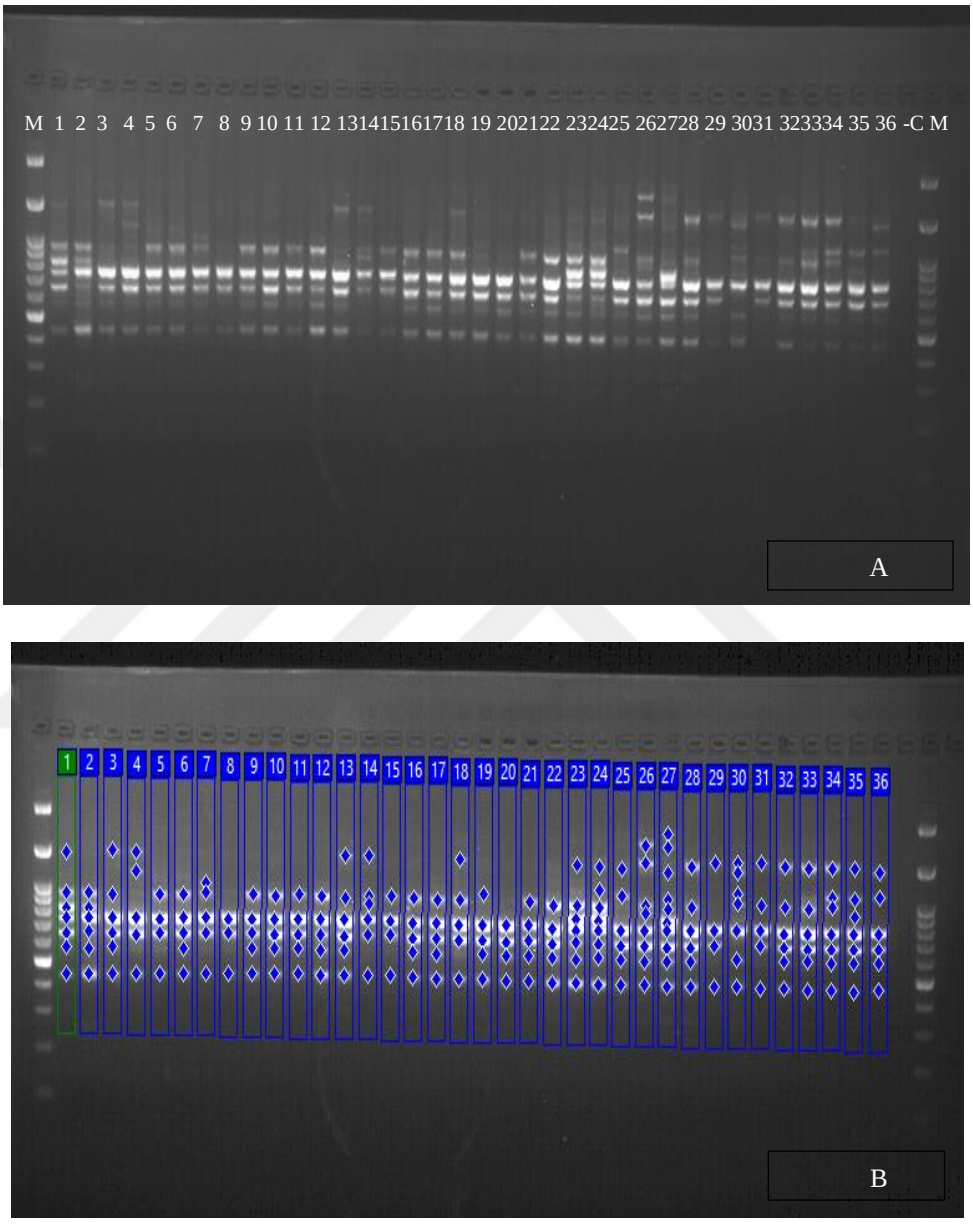


Figure 3.70. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 28 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 32: A total of 21 bands were amplified with primer SCoT 32 (Figure 3.71). The largest band size was 3428 bp and the smallest band was 400 bp, the polymorphism percentage of the primer was 95 % and PIC was calculated as 0.23.

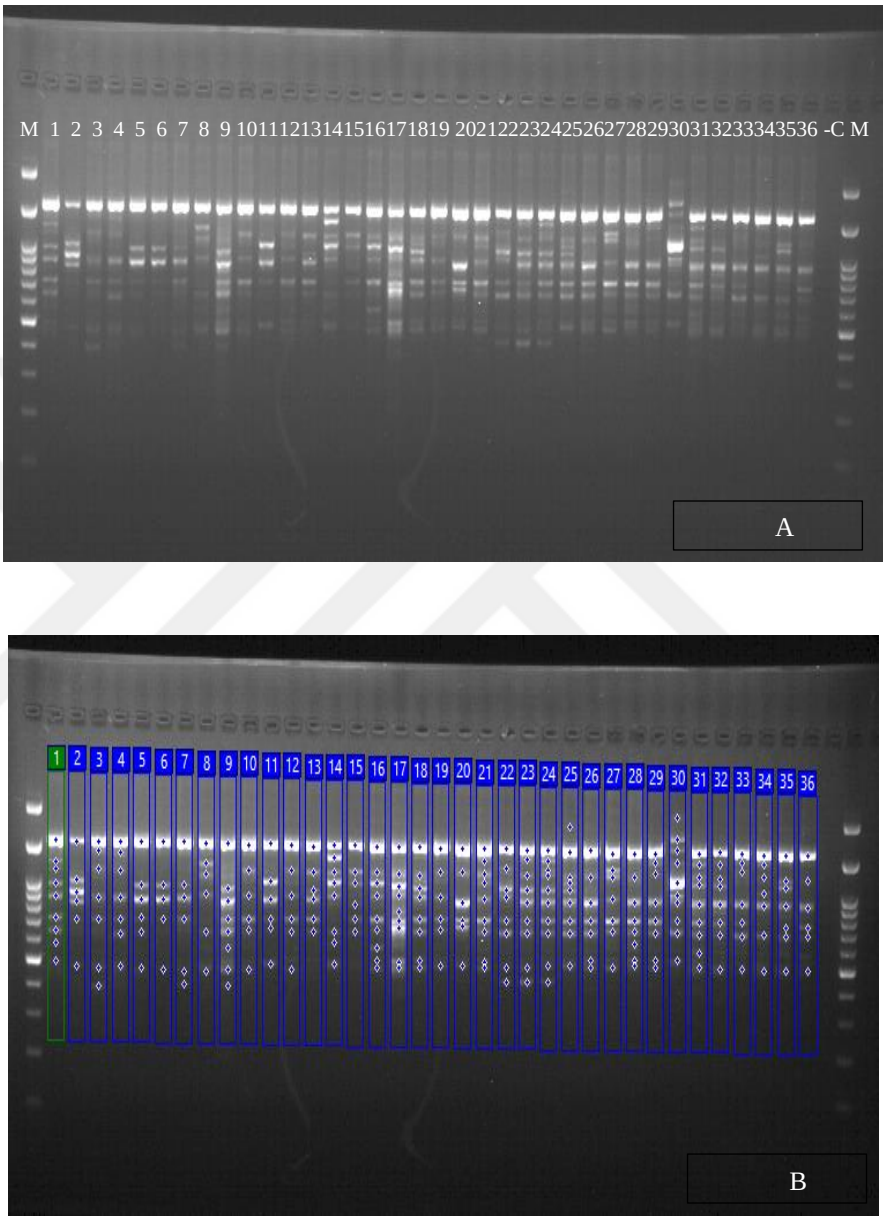


Figure 3.71. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 32 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

SCoT 34: A total of 18 bands were amplified with primer SCoT 34 (Figure 3.72). The largest band size was 3187 bp and the smallest band was 419 bp, the polymorphism percentage of the primer was 94 % and PIC was calculated as 0.31.

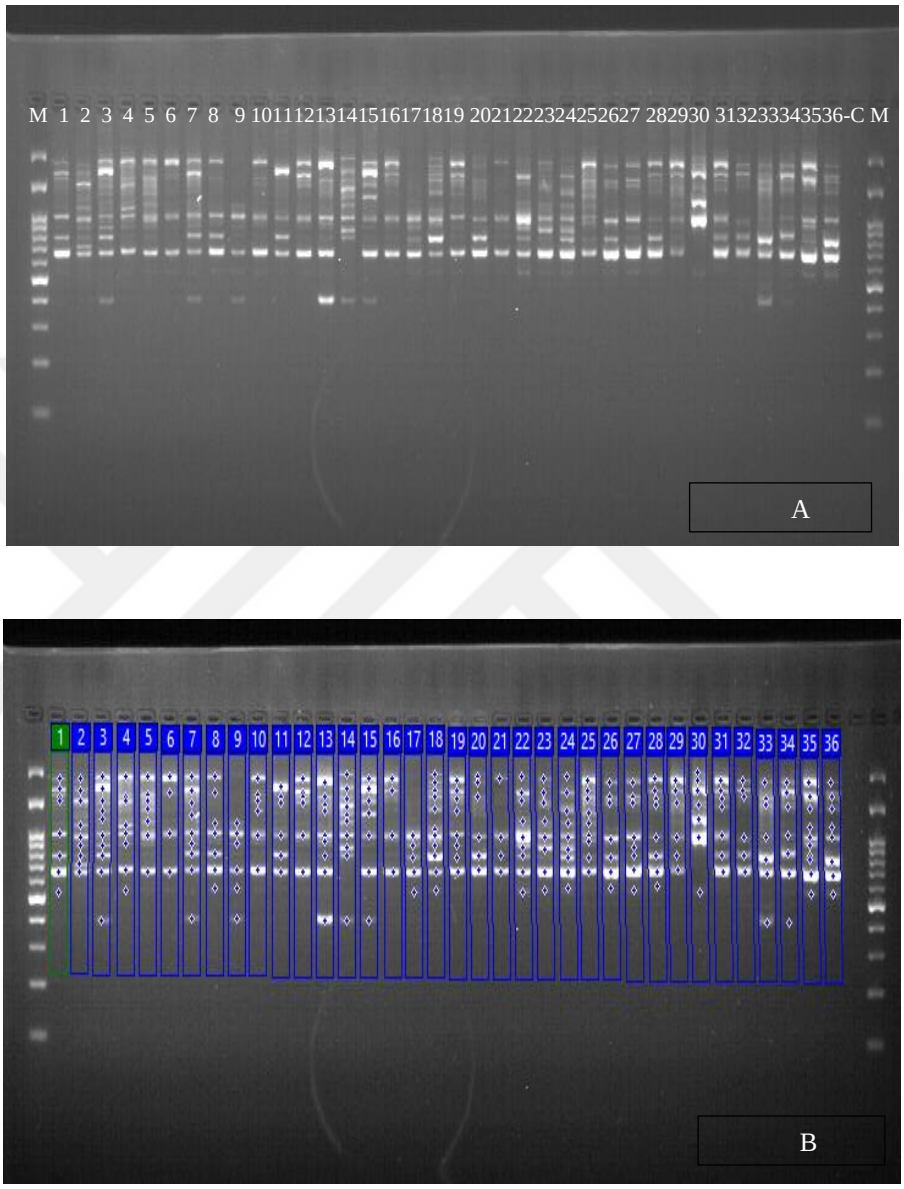


Figure 3.72. *PCR results showing band patterns obtained from 36 almond accessions (1-36) with primer SCoT 34 (A). Band detected with TotalLab Cliqs program (B). M: 100bp plus DNA marker. -C: negative control.*

3.7. Data Analysis of Chickpea Accessions

3.7.1. Assessment of ISSR analysis

ISSR analysis banding patterns showed that 13 primers amplified reproducible banding patterns. A total of 135 bands were detected from 18 chickpea accessions among these bands 110 were polymorphic. The number of amplified bands varied between 2 (ISSR 817) and 18 (ISSR 811) with an average of 10.4 bands per primer. The band sizes ranged from 333 bp (ISSR 811) to 3919 bp (ISSR 811). The polymorphism percentages ranged from 0 to 100 % with an average of 72 %. In addition, the Polymorphism Information Content (PIC) ranged from 0 (ISSR 817) to 0.3 (ISSR 818) with an average of 0.17 (Table 3.10).

Table 3.11. *ISSR analysis of band patterns for chickpea accessions*

Primer	Band Size (bp)	TB	MB	PB	PPB %	PIC
ISSR 807	566-1790	10	3	7	70	0.17
ISSR 810	467-2231	8	1	7	87.5	0.2
ISSR 811	333-3919	18	1	17	94	0.26
ISSR 812	560-1700	9	3	6	67	0.23
ISSR 813	406-1560	4	2	2	50	0.13
ISSR 817	949-1320	2	2	0	0	0
ISSR 818	609-2381	14	0	14	100	0.3
ISSR 819	712-1423	5	2	3	60	0.05
ISSR 829	510-3000	16	1	15	94	0.27
ISSR 834	364-1122	13	1	12	92	0.17
ISSR 847	582-2368	13	3	10	77	0.14
ISSR 853	400-1433	13	3	10	77	0.18
ISSR 855	470-1887	10	3	7	70	0.1
	SUM	135	25	110	-	-
	AVG	10.4	-	-	72	0.17

TB: Total number of bands, MB: Monomorphic Bands, PB: Polymorphic Bands, PPB%: Percentage of Polymorphic Bands, PIC: Polymorphism Information Content, AVG: Average.

The genetic similarity values between chickpea accessions were calculated based on Jaccard's similarity coefficient presented in the DendroUPGMA program. The genetically closest accessions were found as accessions 4537 and 4361 from Maan with a genetic similarity coefficient of 0.769. The lowest genetic similarity was found between accessions 2798 from Madaba and 2795 from Zarqa with a genetic similarity coefficient of 0.452 (Table 3.11).

Table 3.12. *ISSR genetic similarity matrix between chickpea accessions based on Jaccard similarity coefficient.*

	C1 Amman	C4 Amman	C2 Balqa	C3 Mafraq	C5 Maan	C8 Maan	C11 Maan	C17 Maan	C18 Maan
C1 Amman	1	0.634	0.592	0.56	0.62	0.533	0.646	0.556	0.539
C4 Amman		1	0.643	0.676	0.671	0.603	0.603	0.676	0.587
C2 Balqa			1	0.634	0.629	0.629	0.631	0.657	0.657
C3 Mafraq				1	0.686	0.573	0.571	0.62	0.558
C5 Maan					1	0.681	0.662	0.766	0.639
C8 Maan						1	0.612	0.687	0.662
C11 Maan							1	0.694	0.692
C17 Maan								1	0.769
C18 Maan									1
C7 Jerash									
C9 Karak									
C10 Zarqa									
C13 Irbid									
C14 Unknown									
C15 Unknown									
C16 Unknown									
C6 Unknown									
C12 Madaba									

C7 Jerash	C9 Karak	C10 Zarqa	C13 Irbid	C14 Unknown	C15 Unknown	C16 Unknown	C6 Unknown	C12 Madaba
0.541	0.592	0.507	0.527	0.521	0.545	0.5	0.566	0.551
0.634	0.618	0.533	0.62	0.548	0.613	0.569	0.635	0.603
0.638	0.6	0.514	0.623	0.571	0.662	0.618	0.662	0.656
0.581	0.55	0.547	0.547	0.541	0.605	0.52	0.584	0.618
0.643	0.671	0.562	0.606	0.556	0.69	0.556	0.714	0.688
0.62	0.584	0.481	0.606	0.514	0.644	0.556	0.667	0.714
0.646	0.52	0.582	0.606	0.486	0.623	0.552	0.623	0.587
0.697	0.63	0.542	0.632	0.557	0.721	0.627	0.671	0.694
0.603	0.55	0.506	0.568	0.52	0.649	0.629	0.605	0.618
1	0.551	0.527	0.614	0.5	0.63	0.563	0.653	0.621
	1	0.538	0.644	0.616	0.595	0.513	0.556	0.562
		1	0.577	0.467	0.513	0.467	0.494	0.452
			1	0.549	0.639	0.507	0.573	0.559
				1	0.526	0.479	0.526	0.552
					1	0.589	0.653	0.672
						1	0.547	0.576
							1	0.6
								1

An UPGMA dendrogram showing the genetic relationships between the chickpea accessions was obtained using the MEGA 11 software. The UPGMA clustering pattern from ISSR analysis grouped the 18 chickpea accessions into two main clusters: cluster I included only one variety (2795) from Zarqa, and cluster II divided into two sub clusters, the first sub cluster included accessions 2801 from Unknown locality, and the second sub cluster included all the other 16 chickpea accessions divided into smaller groups (Fig. 3.73).

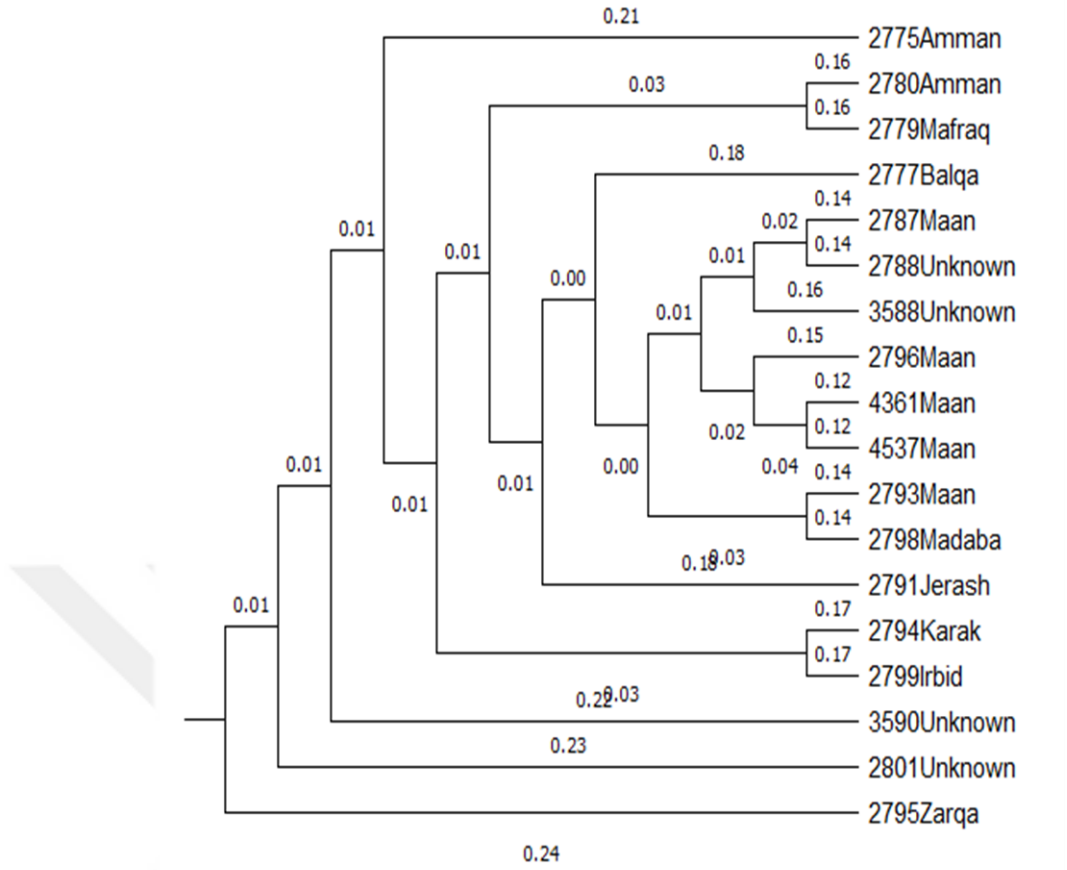


Figure 3.73. *UPGMA clustering pattern of 18 chickpea accessions based on the genetic similarity values of ISSR PCR analyses.*

The genetic structure of the 18 chickpea accessions was performed using the STRUCTURE software clustering model. The software assumed a model of admixture degree for each individual plant and the populations (K) that contribute to the genotype of each individual. The analysis was performed with K = 1-10, each with 10 iterations (Table 3.12). The results showed the highest peak at K=3 indicating the presence of three major clusters (Fig. 3.74 and Fig. 3.75).

Table 3.13. *Average mean values of K=1-10 for chickpea accessions based on ISSR marker.*

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-874.740000	2.110924	—	—	—
2	10	-865.740000	14.593240	9.000000	9.600000	0.657839
3	10	-866.340000	4.527987	-0.600000	38.790000	8.566721
4	10	-905.730000	15.396829	-39.390000	21.960000	1.426268
5	10	-923.160000	23.380866	-17.430000	14.880000	0.636418
6	10	-925.710000	38.886400	-2.550000	36.070000	0.927574
7	10	-892.190000	34.386155	33.520000	35.090000	1.020469
8	10	-893.760000	19.100971	-1.570000	6.180000	0.323544
9	10	-889.150000	16.749411	4.610000	6.830000	0.407776
10	10	-891.370000	19.388545	-2.220000	—	—

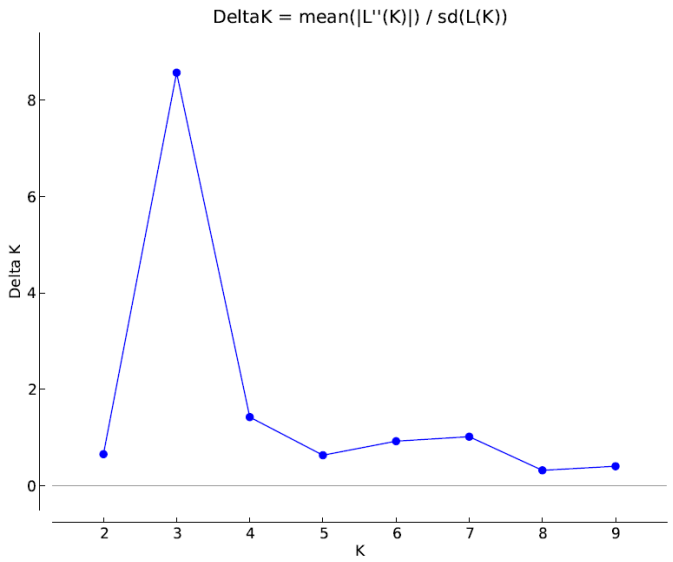


Figure 3.74. *STRUCTURE analysis for the number of populations (K = 3) of chickpea accessions based on ISSR analyses.*

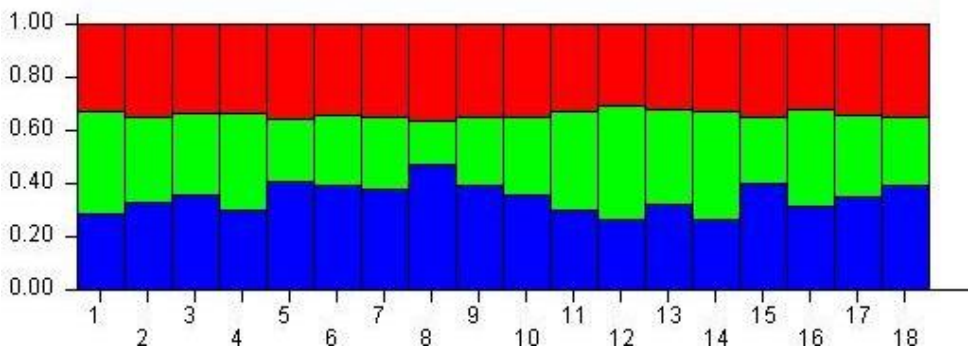


Figure 3.75. *STRUCTURE analysis of chickpea accessions based on ISSR analyses.*

3.7.2. Assessment of SCoT Analysis

SCoT PCR analyses showed that 10 primers amplified reproducible banding patterns, scored as present (1) or absent (0), which was treated as independent character, a binary matrix was produced using phoretix 1D Pro software. A total of 166 bands were detected from 18 chickpea accessions, the number of bands varied between 8 (SCoT 3) and 25 (SCoT 32) with an average of 16.6 bands per primer. Among these bands 129 were polymorphic, the polymorphism percentage ranged from 50 to 92% with an average polymorphism of 75%. The band sizes ranged between 185 bp (SCoT 12) and 3825 bp (SCoT 19). In addition, PIC values ranged from 0.13 (SCoT 18) to 0.35 (SCoT 1) with an average of 0.26 (Table 3.13).

Table 3.14. *SCoT analysis of band patterns for chickpea accessions.*

Primer	Band Size (bp)	TB	MB	PB	PPB %	PIC
SCoT 1	588-2650	13	1	12	92	0.35
SCoT 3	800-2935	8	4	4	50	0.19
SCoT 12	185-2729	20	4	16	80	0.26
SCoT 15	225-2911	21	3	18	86	0.31
SCoT 16	288-3404	20	2	18	90	0.30
SCoT 18	227-2927	13	6	7	54	0.13
SCoT 19	688-3825	17	2	15	88	0.31
SCoT 21	230-1733	14	4	10	71	0.21
SCoT 28	295-3048	15	6	9	60	0.24
SCoT 32	240-3281	25	5	20	80	0.26
	SUM	166	37	129	-	-
	AVG	16.6	-	-	75	0.26

TB: Total number of bands, MB: Monomorphic Bands, PB: Polymorphic Bands, PPB: Percentage of Polymorphic Bands, PIC: Polymorphism Information Content, AVG: Average.

The genetic similarity values between chickpea accessions were calculated based on Jaccard's similarity coefficient presented in the DendroUPGMA program. The genetically closest accessions were accessions 2779 from Mafraq and 2777 from Balqa with a genetic similarity coefficient of 0.744. The lowest genetic similarity was found between accessions 3590 from Unknown locality and 2779 from Mafraq with a genetic similarity coefficient of 0.468 (Table 3.14).

Table 3.15. *SCoT genetic similarity matrix between chickpea accessions based on Jaccard similarity coefficient.*

	2775Amman	2780Amman	2777Balqa	2779Mafraq	2787Maan	2793Maan	2796Maan	4361Maan
2775Amman	1	0.698	0.646	0.653	0.59	0.518	0.643	0.56
2780Amman		1	0.63	0.67	0.654	0.549	0.66	0.636
2777Balqa			1	0.744	0.59	0.518	0.66	0.518
2779Mafraq				1	0.644	0.579	0.649	0.536
2787Maan					1	0.627	0.735	0.642
2793Maan						1	0.648	0.625
2796Maan							1	0.713
4361Maan								1
4537Maan								
2791Jerash								
2794Karak								
2795Zarqa								
2798Madaba								
2799Irbid								
2801Unknown								
3588Unknown								
3590Unknown								
2788Unknown								

4537Maan	2791Jerash	2794Karak	2795Zarqa	2798Madaba	2799Irbid	2801Unknown	3588Unknown	3590Unknown	2788Unknown
0.518	0.533	0.559	0.625	0.545	0.523	0.589	0.496	0.519	0.571
0.633	0.565	0.604	0.611	0.562	0.569	0.606	0.5	0.495	0.59
0.491	0.505	0.504	0.565	0.532	0.551	0.56	0.565	0.519	0.54
0.564	0.552	0.564	0.615	0.565	0.514	0.565	0.527	0.468	0.561
0.655	0.617	0.596	0.604	0.613	0.606	0.642	0.575	0.532	0.583
0.595	0.6	0.568	0.56	0.583	0.508	0.517	0.574	0.491	0.551
0.676	0.59	0.615	0.654	0.663	0.625	0.587	0.578	0.577	0.635
0.682	0.571	0.581	0.56	0.556	0.575	0.611	0.484	0.587	0.596
1	0.627	0.593	0.628	0.529	0.602	0.581	0.508	0.615	0.641
	1	0.598	0.62	0.558	0.593	0.558	0.562	0.532	0.684
		1	0.688	0.623	0.616	0.623	0.559	0.492	0.579
			1	0.574	0.609	0.534	0.593	0.55	0.618
				1	0.618	0.556	0.574	0.504	0.551
					1	0.575	0.595	0.61	0.588
						1	0.56	0.545	0.596
							1	0.55	0.528
								1	0.602
									1

An UPGMA dendrogram showing the genetic relationships between the chickpea accessions was obtained using the MEGA 11 software. The UPGMA clustering pattern from SCoT marker analyses grouped the 18 chickpea accessions into two main clusters: cluster I included accessions 3590 and 3588 from Unknown localities and cluster II divided into two sub clusters; subcluster I included accessions 2801 (Unknown), 2799 (Irbid), 2798 (Madaba), 2795 (Zarqa), 2794 (Karak); 2788 (Unknown), 2791 (Jerash), 2793, 4537, 4361, 2796 and 2787 from (Maan), while sub cluster II included 2779 (Mafraq), 2777 (Balqa), 2780 and 2775 from (Amman) accessions (Fig. 3.76).

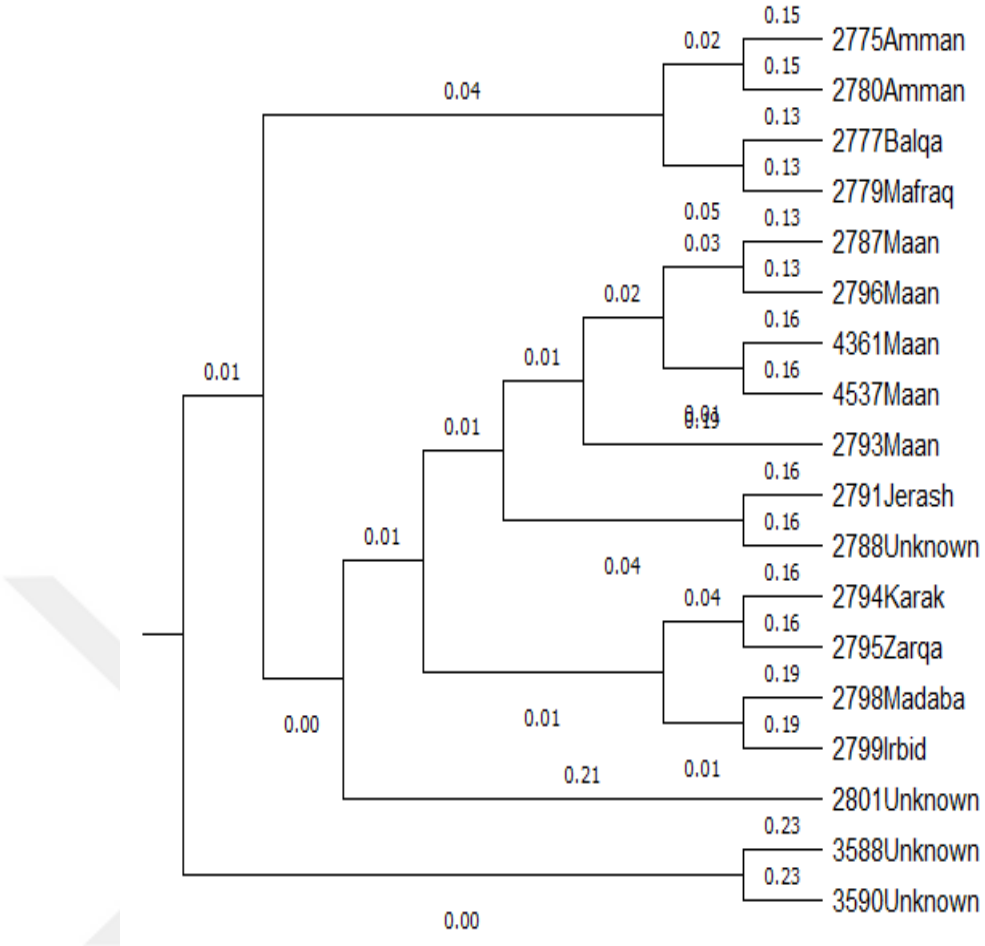


Figure 3.76. *UPGMA clustering pattern of 18 chickpea accessions based on the genetic similarity values of SCoT PCR analyses.*

The genetic structure of the 18 chickpea accessions was determined using the STRUCTURE software clustering model. The software assumed a model of admixture degree for each individual plant and the populations (K) that contribute to the genotype of each individual. The analysis was performed with K = 1-10, each with 10 iterations (Table 3.15). The results showed the highest peak at K=3 indicating the presence of three major clusters (Fig. 3.77 and Fig. 3.78).

Table 3.16. *Average mean values of K=1-10 for chickpea accessions based on SCoT analyses.*

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-1276.090000	1.372305	—	—	—
2	10	-1352.580000	154.593645	-76.490000	126.560000	0.818662
3	10	-1302.510000	32.951967	50.070000	248.790000	7.550080
4	10	-1501.230000	283.957591	-198.720000	252.180000	0.888090
5	10	-1447.770000	101.058180	53.460000	201.280000	1.991724
6	10	-1595.590000	366.995315	-147.820000	116.570000	0.317633
7	10	-1626.840000	422.567626	-31.250000	84.640000	0.200299
8	10	-1573.450000	358.273670	53.390000	192.360000	0.536908
9	10	-1712.420000	405.661917	-138.970000	6.900000	0.017009
10	10	-1844.490000	407.525662	-132.070000	—	—

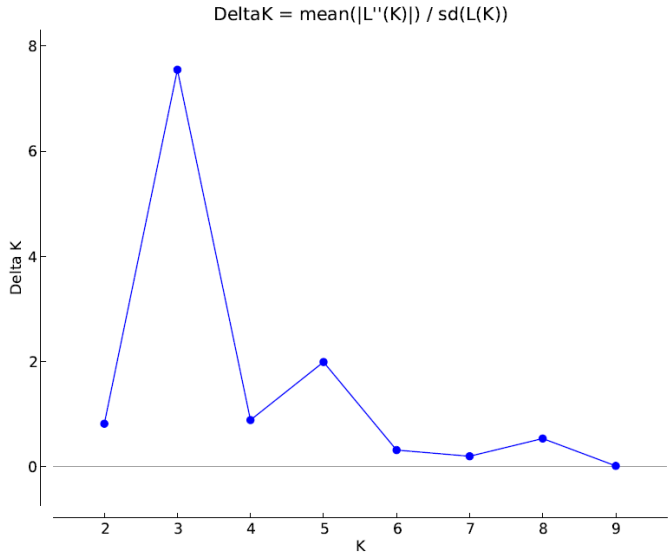


Figure 3.77. *STRUCTURE analysis for the number of populations (K =3) of chickpea accessions based on SCoT analyses.*

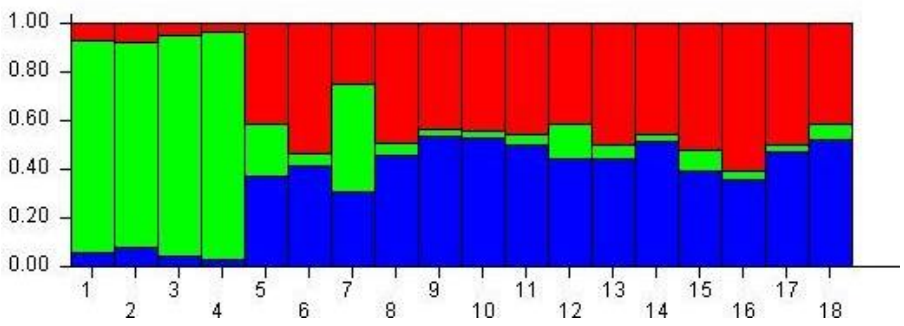


Figure 3.78. *STRUCTURE analysis of chickpea accessions based on SCoT analyses.*

3.8. Data Analysis of Lentil Accessions

3.8.1. Assessment of ISSR analysis

ISSR analysis results showed that 13 primers amplified reproducible banding patterns. A total of 170 bands were detected from Lentil accessions, among these bands 157 were polymorphic. The number of amplified bands varied between 8 (ISSR 813) and 20 (ISSR 808) with an average of 13 bands per primer. The band sizes ranged from 306 bp (ISSR 834) to 2887 bp (ISSR 819). The polymorphism percentage ranged from 83 to 100% with an average polymorphism of 92%. In addition, the PIC values were ranged from 0.1 (ISSR 813) to 0.33 (ISSR 855) with an average of 0.24 (Table 3.16).

Table 3.17. *ISSR analysis of band patterns for lentil accessions.*

Primer	Band Size (bp)	TB	MB	PB	PPB %	PIC
ISSR 807	882-2210	12	2	10	83	0.19
ISSR 808	423-2088	20	1	19	95	0.3
ISSR 809	454-1737	18	2	16	89	0.26
ISSR 812	858-2288	10	1	9	90	0.16
ISSR 813	700-1619	8	1	7	87.5	0.1
ISSR 814	570-2000	11	1	10	91	0.2
ISSR 817	800-2460	10	1	9	90	0.26
ISSR 819	611-2887	15	1	14	93	0.3
ISSR 824	391-1640	10	0	10	100	0.3
ISSR 834	306-2209	15	0	15	100	0.31
ISSR 847	563-2160	16	1	15	94	0.21
ISSR 853	534-1739	11	1	10	91	0.25
ISSR 855	611-2412	14	1	13	93	0.33
	SUM	170	13	157	-	-
	AVG	13	-	-	92	0.24

TB: Total number of bands, MB: Monomorphic Bands, PB: Polymorphic Bands, PPB%: Percentage of Polymorphic Bands, PIC: Polymorphism Information Content, AVG: Average.

The genetic similarity values between 35 Lentil (27 cultivated and 8 wild (LE)) accessions were calculated based on Jaccard's similarity coefficient presented in the UPGMA Dendrogram program. The genetically closest accessions within the 35 accessions were between accessions (LE 3428) from Irbid and (LE 3426) from Balqa with a genetic similarity coefficient of 0.875. The lowest genetic similarity was found between (LE 3426) from Balqa and (L 3380) from Balqa with a genetic similarity coefficient of 0.176. Comparing the cultivated *L. culinaris* accessions with each other, the genetically closest accessions were found between 2 accessions from Karak (L 3409) and (L 3395)

with a genetic similarity coefficient of 0.826, while the lowest genetic similarity was found between accessions (L 4362) from Maan and (L 3363) from Jerash with a genetic similarity coefficient of 0.463. Comparing the *L. ervoides* accessions with each other, the genetically closest accessions were found between (LE 3428) from Irbid and (LE 3426) from Balqa with a genetic similarity coefficient of 0.875, while the lowest genetic similarity was found between (LE 3437) from Karak and (LE 3412) from Ajlun with a genetic similarity coefficient of 0.240 (Table 3.17).

Table 3.18. *ISSR genetic similarity matrix between lentil accessions based on Jaccard similarity coefficient.*

	L3352Irbid	L3360Irbid	L3384Irbid	L3388Irbid	L3400Irbid	L3401Irbid	L3353Jerash	L3362Jerash
L3352Irbid	1	0.729	0.667	0.658	0.693	0.694	0.69	0.584
L3360Irbid		1	0.636	0.627	0.641	0.662	0.658	0.618
L3384Irbid			1	0.68	0.692	0.628	0.562	0.494
L3388Irbid				1	0.753	0.685	0.592	0.537
L3400Irbid					1	0.817	0.628	0.554
L3401Irbid						1	0.605	0.57
L3353Jerash							1	0.718
L3362Jerash								1
L3363Jerash								
L3364Ajlun								
L3379Ajlun								
L3404Ajlun								
L4339Ajlun								
L3365Amman								
L3366Madaba								
L3370Madaba								
L3374Madaba								
L3376Balqa								
L3377Balqa								
L3380Balqa								
L3390Karak								
L3392Karak								
L3395Karak								
L3409Karak								
L3411Tafila								
L4362Maan								
L4538Maan								
LE3412Ajlun								
LE3426Balqa								
LE3428Irbid								
LE3429Irbid								
LE3435Maan								
LE3436Tafila								
LE3437Karak								
LE3438Tafila								

Table 3.17. (Continued) *ISSR genetic similarity matrix between lentil accessions based on Jaccard similarity coefficient.*

L3363Jerash	L3364Ajlun	L3379Ajlun	L3404Ajlun	L4339Ajlun	L3365Amman	L3366Madaba	L3370Madaba
0.676	0.72	0.585	0.718	0.6	0.635	0.662	0.635
0.623	0.711	0.617	0.685	0.635	0.584	0.61	0.605
0.573	0.675	0.588	0.789	0.582	0.68	0.641	0.658
0.562	0.646	0.578	0.64	0.592	0.605	0.61	0.564
0.617	0.722	0.612	0.697	0.628	0.6	0.605	0.62
0.575	0.658	0.553	0.632	0.605	0.519	0.562	0.577
0.746	0.675	0.605	0.627	0.644	0.729	0.757	0.754
0.615	0.578	0.483	0.531	0.544	0.577	0.645	0.618
1	0.727	0.558	0.658	0.57	0.689	0.764	0.689
	1	0.655	0.701	0.633	0.711	0.671	0.605
		1	0.59	0.646	0.523	0.494	0.598
			1	0.649	0.708	0.667	0.685
				1	0.592	0.557	0.613
					1	0.797	0.694
						1	0.676
							1

L3374Madaba	L3376Balqa	L3377Balqa	L3380Balqa	L3390Karak	L3392Karak	L3395Karak	L3409Karak	L3411Tafila
0.611	0.71	0.667	0.608	0.641	0.676	0.616	0.62	0.603
0.671	0.7	0.589	0.622	0.613	0.622	0.63	0.654	0.573
0.729	0.757	0.714	0.653	0.642	0.699	0.577	0.642	0.544
0.581	0.653	0.657	0.579	0.63β	0.622	0.653	0.633	0.639
0.597	0.645	0.649	0.575	0.667	0.726	0.667	0.688	0.61
0.553	0.622	0.625	0.571	0.646	0.635	0.6	0.605	0.7
0.681	0.639	0.575	0.566	0.58	0.63	0.639	0.641	0.539
0.616	0.538	0.5	0.551	0.548	0.532	0.579	0.566	0.587
0.644	0.584	0.545	0.537	0.59	0.662	0.584	0.61	0.494
0.603	0.628	0.57	0.506	0.612	0.62	0.608	0.631	0.537
0.482	0.561	0.524	0.536	0.568	0.593	0.62	0.605	0.512
0.735	0.765	0.746	0.704	0.711	0.779	0.622	0.667	0.608
0.547	0.595	0.575	0.566	0.684	0.587	0.662	0.641	0.581
0.696	0.7	0.611	0.579	0.573	0.622	0.566	0.613	0.475
0.75	0.681	0.595	0.584	0.617	0.627	0.592	0.658	0.538
0.721	0.676	0.657	0.622	0.593	0.69	0.566	0.613	0.532
1	0.781	0.632	0.62	0.57	0.667	0.562	0.59	0.507
	1	0.738	0.696	0.615	0.721	0.611	0.68	0.575
		1	0.727	0.597	0.727	0.592	0.577	0.623
			1	0.588	0.71	0.603	0.628	0.589
				1	0.693	0.703	0.744	0.582
					1	0.696	0.74	0.568
						1	0.826	0.62
							1	0.562
								1

Table 3.17. (Continued) *ISSR genetic similarity matrix between lentil accessions based on Jaccard similarity coefficient.*

L4362Maan	L4538Maan	LE3412Ajlun	LE3426Balqa	LE3428Irbid	LE3429Irbid	LE3435Maan	LE3436Tafila	LE3437Karak	LE3438Tafila
0.589	0.551	0.253	0.222	0.227	0.419	0.396	0.4	0.386	0.36
0.603	0.584	0.276	0.22	0.224	0.43	0.438	0.412	0.432	0.356
0.551	0.556	0.277	0.212	0.228	0.412	0.435	0.425	0.412	0.385
0.625	0.627	0.263	0.232	0.237	0.4	0.391	0.463	0.415	0.371
0.597	0.62	0.284	0.243	0.26	0.404	0.457	0.448	0.419	0.407
0.616	0.618	0.248	0.218	0.222	0.426	0.433	0.407	0.393	0.382
0.547	0.512	0.265	0.222	0.227	0.404	0.427	0.384	0.386	0.344
0.595	0.519	0.273	0.23	0.222	0.354	0.372	0.375	0.376	0.323
0.463	0.543	0.243	0.202	0.206	0.402	0.394	0.398	0.384	0.374
0.543	0.585	0.267	0.204	0.208	0.439	0.447	0.438	0.409	0.383
0.518	0.541	0.276	0.236	0.24	0.42	0.457	0.449	0.42	0.409
0.573	0.618	0.273	0.206	0.222	0.381	0.418	0.44	0.41	0.414
0.611	0.681	0.253	0.21	0.227	0.419	0.411	0.487	0.386	0.391
0.481	0.488	0.25	0.208	0.212	0.385	0.391	0.395	0.381	0.341
0.526	0.55	0.257	0.216	0.22	0.378	0.383	0.386	0.388	0.333
0.539	0.506	0.263	0.22	0.237	0.4	0.391	0.395	0.398	0.371
0.534	0.539	0.277	0.219	0.237	0.347	0.398	0.402	0.405	0.345
0.606	0.566	0.245	0.214	0.232	0.398	0.404	0.41	0.412	0.384
0.657	0.589	0.214	0.208	0.226	0.411	0.386	0.39	0.41	0.365
0.643	0.558	0.218	0.176	0.192	0.351	0.37	0.388	0.425	0.364
0.61	0.697	0.282	0.24	0.257	0.414	0.467	0.53	0.414	0.402
0.597	0.558	0.255	0.212	0.229	0.337	0.432	0.457	0.407	0.412
0.727	0.7	0.271	0.253	0.272	0.398	0.42	0.539	0.412	0.417
0.653	0.675	0.282	0.24	0.257	0.386	0.406	0.477	0.398	0.418
0.687	0.595	0.235	0.242	0.234	0.387	0.378	0.365	0.333	0.341
1	0.721	0.277	0.258	0.278	0.391	0.367	0.474	0.423	0.36
	1	0.25	0.22	0.237	0.415	0.391	0.519	0.45	0.386
		1	0.812	0.836	0.347	0.26	0.268	0.24	0.276
			1	0.875	0.343	0.267	0.25	0.247	0.258
				1	0.365	0.273	0.269	0.253	0.277
					1	0.404	0.379	0.351	0.43
						1	0.595	0.544	0.506
							1	0.52	0.622
								1	0.568
									1

An UPGMA dendrogram showing the genetic relationships between the lentil accessions was obtained using the MEGA 11 software. The UPGMA clustering pattern from ISSR analyses grouped the 35 lentil accessions into three clusters: cluster I included accessions of *L. ervoides* (LE 3412) from Ajlun, (LE 3426) from Balqa and (LE 3428) from Irbid; cluster II included (LE 3429) from Irbid, (LE 3435) from Maan, (LE 3436) and (LE 3438) from Tafila and (LE 3437) from Karak, while cluster III included all the cultivated *L. culinaris* accessions into smaller groups (Fig. 3.79).

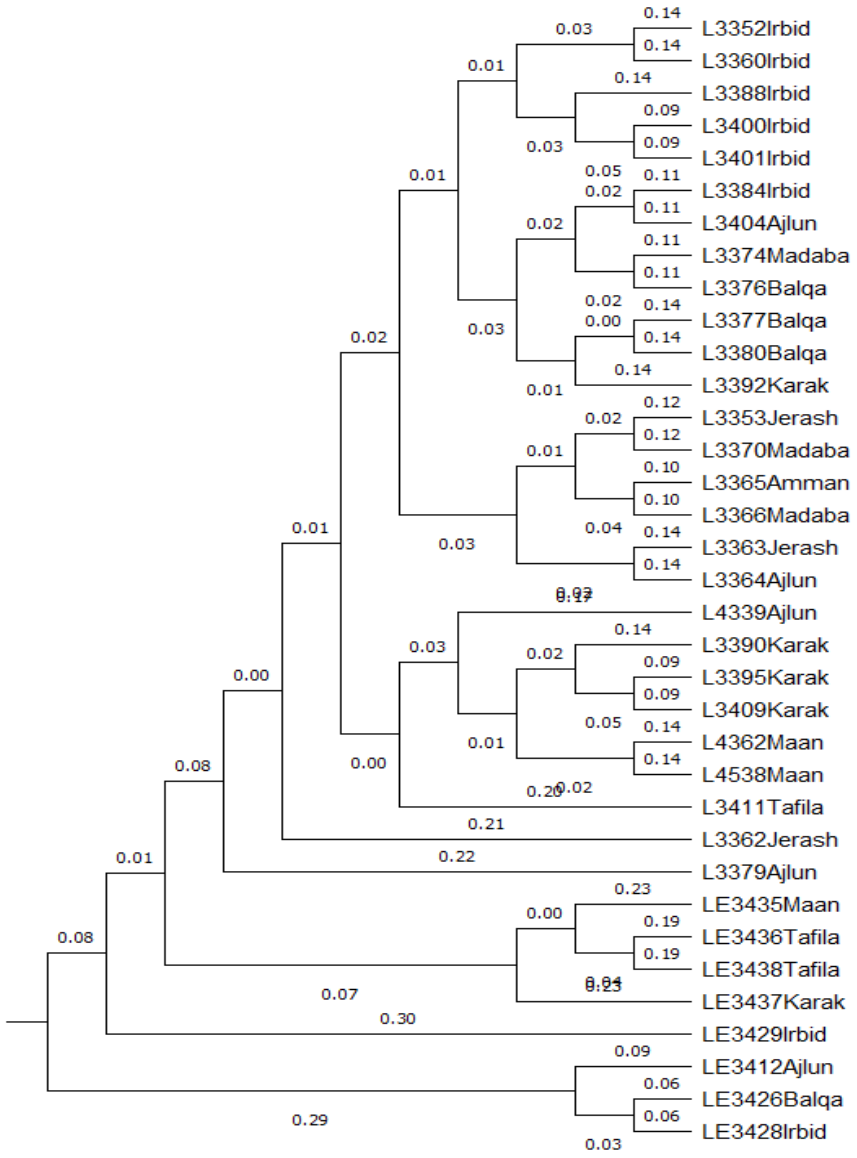


Figure 3.79. *UPGMA clustering pattern of 35 lentil accessions based on the genetic similarity values of ISSR analyses.*

The genetic structure of the 35 lentil accessions were determined using the STRUCTURE software clustering model. The software assumed a model of admixture degree for each individual plant and the populations (K) that contribute to the genotype of each individual. The analysis was performed with K = 1-10, each with 10 iterations (Table 3.18). The results showed the highest peak at K= 2 indicating the presence of two major clusters **(**Fig .3.80 and Fig. 3.81).

Table 3.19. *Average mean values of K=1-10 for lentil accessions based on ISSR analyses.*

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-2371.960000	1.091584	—	—	—
2	10	-1903.050000	2.845367	468.910000	301.000000	105.786017
3	10	-1735.140000	26.308098	167.910000	218.750000	8.314930
4	10	-1785.980000	293.486667	-50.840000	137.190000	0.467449
5	10	-1699.630000	8.444597	86.350000	193.490000	22.912875
6	10	-1806.770000	392.031240	-107.140000	223.390000	0.569827
7	10	-1690.520000	61.707766	116.250000	120.250000	1.948701
8	10	-1694.520000	79.282011	-4.000000	9.270000	0.116924
9	10	-1689.250000	41.610609	5.270000	368.970000	8.867210
10	10	-2052.950000	586.712798	-363.700000	—	—

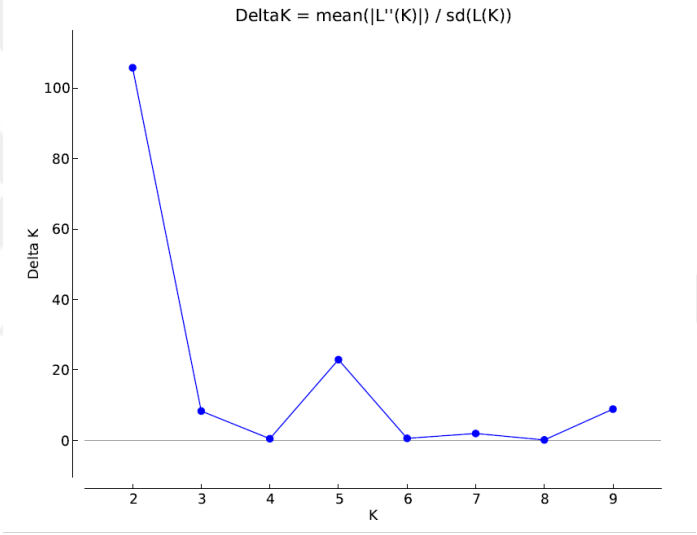


Figure 3.80. *STRUCTURE analysis for the number of populations (K = 2) of lentil accessions based on ISSR analyses.*

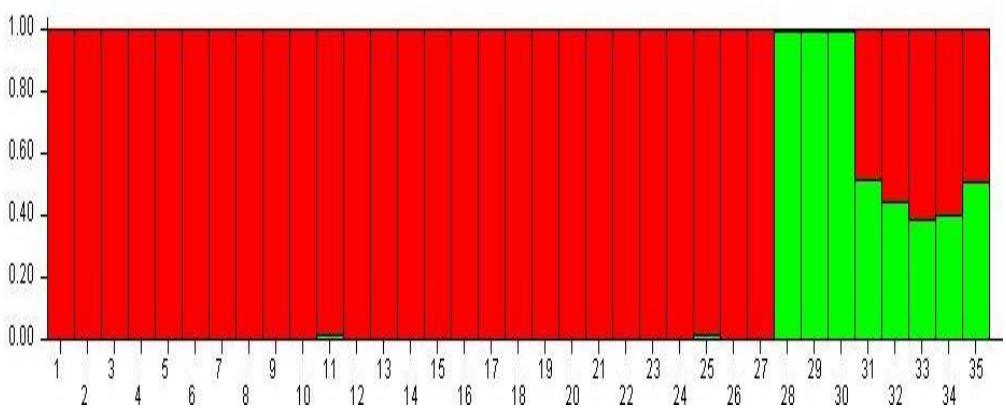


Figure 3.81. *STRUCTURE analysis of lentil accessions based on ISSR analyses.*

3.8.2. Assessment of SCoT Analysis

SCoT PCR analyses results showed that 10 primers amplified reproducible banding patterns. A total of 174 bands were detected from lentil accessions, the number of bands varied between 13 (SCoT 21) and 26 (SCoT 18) with an average of 17.4 bands per primer. Among these bands 155 were polymorphic, the polymorphism percentage ranged from 84 to 100% with an average polymorphism of 89%. The band sizes ranged from 247 bp (SCoT 17) to 3720 bp (SCoT 16). In addition, the PIC values were ranged from 0.18 (SCoT 21) to 0.37 (SCoT 3) with an average of 0.27 (Table 3.19).

Table 3.20. *SCoT analysis of band patterns for lentil accessions*

Primer	Band Size (bp)	TB	MB	PB	PPB %	PIC
SCoT 3	461-2588	20	0	20	100	0.37
SCoT 15	310-2244	16	2	14	88	0.3
SCoT 16	675-3720	19	3	16	84	0.25
SCoT 17	247-1620	19	3	16	84	0.2
SCoT 18	355-2454	26	1	25	96	0.34
SCoT 19	625-2689	16	1	15	94	0.2
SCoT 21	370-2254	13	2	11	85	0.18
SCoT 28	265-2200	14	2	12	86	0.3
SCoT 32	279-2537	15	3	12	80	0.26
SCoT 34	330-3500	16	2	14	88	0.28
	SUM	174	19	155	-	-
	AVG	17.4	-	-	89	0.27

TB: Total number of bands, MB: Monomorphic Bands, PB: Polymorphic Bands, PPB%: Percentage of Polymorphic Bands, PIC: Polymorphism Information Content, AVG: Average.

The genetic similarity values between 35 lentil accessions; 27 cultivated *L. culinaris* and 8 *L. ervoides* (wild) accessions were calculated based on Jaccard's similarity coefficient presented in the DendroUPGMA program. The genetically closest accessions within the 35 accessions were between accessions (LE 3426) from Balqa and (LE 3412) from Ajlun with a genetic similarity coefficient of 0.872. The lowest genetic similarity was found between accessions (LE 3426) and (L 3377) from Balqa with a genetic similarity coefficient of 0.254. Comparing the cultivated *L. culinaris* accessions with each other, the genetically closest ones were found between accessions (L 3374) and (L 3370) from Madaba with a genetic similarity coefficient of 0.831, while the lowest genetic similarity was found between accessions (L 3411) from Tafila and (L 3379) from Ajlun with a genetic similarity coefficient of 0.423. Comparing the

wild *L. ervoides* accessions with each other, the genetically closest accessions were found between (LE 3426) from Balqa and (LE 3412) from Ajlun with a genetic similarity coefficient of 0.872, while the lowest genetic similarity was found between (LE 3436) from Tafila and (LE 3428) from Irbid with a genetic similarity coefficient of 0.297 (Table 3.20).

Table 3.21. *SCoT genetic similarity matrix between lentil accessions based on Jaccard similarity coefficient.*

	L3352Irbid	L3360Irbid	L3384Irbid	L3388Irbid	L3400Irbid	L3401Irbid	L3353Jerash	L3362Jerash
L3352Irbid	1	0.718	0.663	0.663	0.677	0.628	0.659	0.585
L3360Irbid		1	0.697	0.72	0.674	0.641	0.656	0.652
L3384Irbid			1	0.741	0.713	0.717	0.642	0.638
L3388Irbid				1	0.756	0.701	0.678	0.694
L3400Irbid					1	0.731	0.691	0.619
L3401Irbid						1	0.642	0.604
L3353Jerash							1	0.708
L3362Jerash								1
L3363Jerash								
L3364Ajlun								
L3379Ajlun								
L3404Ajlun								
L4339Ajlun								
L3365Amman								
L3366Madaba								
L3370Madaba								
L3374Madaba								
L3376Balqa								
L3377Balqa								
L3380Balqa								
L3390Karak								
L3392Karak								
L3395Karak								
L3409Karak								
L3411Tafila								
L4362Maan								
L4538Maan								
LE3412Ajlun								
LE3426Balqa								
LE3428Irbid								
LE3429Irbid								
LE3435Maan								
LE3436Tafila								
LE3437Karak								
LE3438Tafila								

Table 3.20. (Continued) *SCoT genetic similarity matrix between lentil accessions based on Jaccard similarity coefficient.*

L3363Jerash	L3364Ajlun	L3379Ajlun	L3404Ajlun	L4339Ajlun	L3365Amma	L3366Madab	L3370Madab	L3374Madab
0.591	0.552	0.558	0.649	0.582	0.585	0.63	0.721	0.689
0.64	0.598	0.604	0.681	0.577	0.633	0.644	0.759	0.685
0.645	0.656	0.681	0.72	0.6	0.638	0.632	0.681	0.707
0.682	0.636	0.682	0.724	0.63	0.674	0.667	0.702	0.67
0.576	0.67	0.677	0.698	0.646	0.635	0.646	0.677	0.667
0.594	0.638	0.645	0.702	0.702	0.571	0.667	0.645	0.602
0.659	0.67	0.557	0.663	0.596	0.652	0.681	0.697	0.685
0.795	0.613	0.536	0.592	0.592	0.613	0.697	0.693	0.681
1	0.637	0.558	0.649	0.598	0.674	0.724	0.682	0.727
	1	0.62	0.696	0.66	0.613	0.716	0.656	0.663
		1	0.742	0.649	0.656	0.63	0.609	0.583
			1	0.723	0.714	0.725	0.722	0.71
				1	0.642	0.707	0.667	0.622
					1	0.697	0.62	0.663
						1	0.786	0.812
							1	0.831
								1

L3376Balqa	L3377Balqa	L3380Balqa	L3390Karak	L3392Karak	L3395Karak	L3409Karak	L3411Tafila	L4362Maan
0.67	0.602	0.651	0.556	0.656	0.617	0.571	0.516	0.606
0.596	0.581	0.646	0.634	0.709	0.648	0.567	0.478	0.536
0.725	0.73	0.69	0.674	0.75	0.67	0.639	0.43	0.515
0.709	0.674	0.671	0.733	0.735	0.75	0.637	0.529	0.57
0.684	0.619	0.576	0.67	0.688	0.758	0.62	0.505	0.606
0.635	0.604	0.614	0.606	0.674	0.707	0.639	0.49	0.56
0.685	0.617	0.647	0.619	0.689	0.667	0.635	0.533	0.588
0.611	0.648	0.663	0.667	0.63	0.628	0.582	0.562	0.583
0.652	0.656	0.631	0.621	0.656	0.6	0.588	0.516	0.557
0.681	0.648	0.586	0.615	0.63	0.628	0.632	0.433	0.52
0.634	0.585	0.505	0.656	0.656	0.652	0.604	0.423	0.48
0.747	0.66	0.6	0.66	0.733	0.691	0.66	0.48	0.564
0.674	0.625	0.565	0.594	0.625	0.622	0.677	0.465	0.58
0.663	0.667	0.586	0.703	0.63	0.663	0.582	0.479	0.52
0.711	0.659	0.616	0.66	0.716	0.711	0.66	0.505	0.594
0.689	0.602	0.651	0.638	0.774	0.689	0.674	0.533	0.641
0.753	0.681	0.64	0.663	0.759	0.677	0.646	0.479	0.598
1	0.779	0.699	0.612	0.7	0.696	0.646	0.511	0.55
	1	0.725	0.649	0.667	0.628	0.632	0.463	0.52
		1	0.607	0.605	0.64	0.589	0.494	0.538
			1	0.703	0.717	0.739	0.469	0.495
				1	0.739	0.742	0.479	0.567
					1	0.699	0.527	0.582
						1	0.5	0.554
							1	0.699
								1

Table 3.20. (Continued) *SCoT genetic similarity matrix between lentil accessions based on Jaccard similarity coefficient.*

L4538Maan	LE3412Ajlun	LE3426Balqa	LE3428Irbid	LE3429Irbid	LE3435Maan	LE3436Tafila	LE3437Karak	LE3438Tafila
0.549	0.308	0.299	0.308	0.411	0.467	0.423	0.441	0.429
0.529	0.303	0.261	0.28	0.49	0.49	0.404	0.45	0.423
0.567	0.306	0.276	0.306	0.5	0.544	0.417	0.448	0.45
0.594	0.31	0.267	0.298	0.505	0.536	0.444	0.479	0.467
0.612	0.331	0.311	0.331	0.486	0.558	0.444	0.476	0.48
0.583	0.306	0.287	0.306	0.486	0.5	0.43	0.421	0.45
0.61	0.322	0.303	0.322	0.481	0.524	0.48	0.531	0.49
0.639	0.295	0.254	0.273	0.476	0.52	0.447	0.451	0.469
0.612	0.319	0.267	0.264	0.51	0.54	0.495	0.47	0.505
0.639	0.317	0.286	0.305	0.567	0.566	0.505	0.51	0.533
0.549	0.319	0.299	0.308	0.541	0.495	0.465	0.413	0.474
0.602	0.344	0.303	0.333	0.477	0.505	0.409	0.439	0.455
0.684	0.344	0.314	0.322	0.534	0.519	0.476	0.467	0.485
0.622	0.35	0.297	0.316	0.476	0.476	0.433	0.437	0.454
0.616	0.314	0.273	0.292	0.5	0.529	0.515	0.49	0.511
0.629	0.319	0.299	0.308	0.466	0.495	0.465	0.47	0.458
0.604	0.309	0.279	0.298	0.462	0.505	0.49	0.495	0.485
0.604	0.298	0.268	0.298	0.49	0.519	0.49	0.495	0.485
0.574	0.295	0.254	0.283	0.52	0.505	0.461	0.465	0.484
0.564	0.315	0.27	0.303	0.489	0.49	0.412	0.447	0.466
0.608	0.347	0.295	0.303	0.51	0.495	0.439	0.457	0.46
0.559	0.317	0.275	0.283	0.476	0.49	0.447	0.465	0.439
0.604	0.353	0.322	0.353	0.535	0.534	0.476	0.48	0.455
0.64	0.315	0.274	0.282	0.524	0.538	0.495	0.515	0.49
0.558	0.324	0.279	0.288	0.396	0.426	0.408	0.412	0.413
0.695	0.345	0.336	0.345	0.481	0.51	0.452	0.471	0.444
1	0.347	0.339	0.336	0.519	0.533	0.491	0.481	0.5
	1	0.872	0.761	0.404	0.37	0.319	0.333	0.38
		1	0.847	0.384	0.35	0.333	0.325	0.385
			1	0.381	0.347	0.297	0.322	0.355
				1	0.653	0.659	0.613	0.607
					1	0.73	0.719	0.738
						1	0.771	0.728
							1	0.716
								1

An UPGMA dendrogram showing the genetic relationships between the lentil accessions was obtained using the MEGA 11 software. The UPGMA clustering pattern from SCoT PCR analyses grouped the Lentil accessions into three clusters. Clusters I and II contained all *L. ervoides* accessions, cluster I included (LE 3412) from Ajlun, (LE 3426) from Balqa and (LE 3428) from Irbid, cluster II included (LE 3429) from Irbid, (LE 3435) from Maan, (LE 3436) and (LE 3438) from Tafila and (LE 3437) from Karak, and cluster III included all the cultivated *L. culinaris* accessions divided into smaller groups (Fig. 3.82).

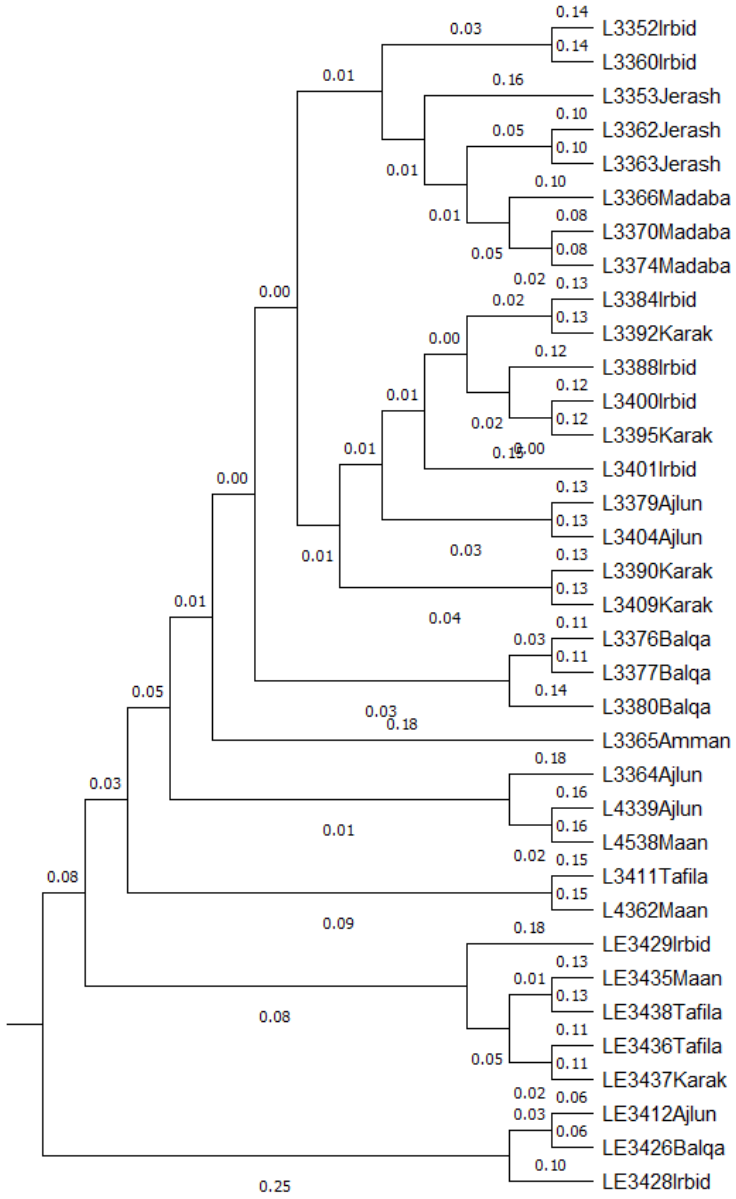


Figure 3.82. *UPGMA clustering pattern of 35 lentil accessions based on the genetic similarity of SCoT analyses.*

The genetic structure of the 35 lentil accessions was determined using the STRUCTURE software clustering model. The software assumed a model of admixture degree for each individual plant and the populations (K) that contribute to the genotype of each individual. The analysis was performed with K = 1-10, each with 10 iterations (Table 3.21). The results showed the highest peak at K= 3 indicating the presence of three major clusters (Fig .3.83 and Fig. 3.84).

Table 3.22. *Average mean values of K=1-10 for lentil accessions based on SCoT analyses.*

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-2511.960000	0.838252	—	—	—
2	10	-2223.720000	464.309269	288.240000	129.050000	0.277940
3	10	-1806.430000	6.193375	417.290000	428.030000	69.110950
4	10	-1817.170000	161.477649	-10.740000	98.450000	0.609682
5	10	-1926.360000	449.194625	-109.190000	244.540000	0.544397
6	10	-1791.010000	150.883770	135.350000	89.310000	0.591913
7	10	-1744.970000	6.040245	46.040000	51.890000	8.590712
8	10	-1750.820000	21.847542	-5.850000	15.780000	0.722278
9	10	-1740.890000	5.656550	9.930000	17.820000	3.150330
10	10	-1748.780000	25.032015	-7.890000	—	—

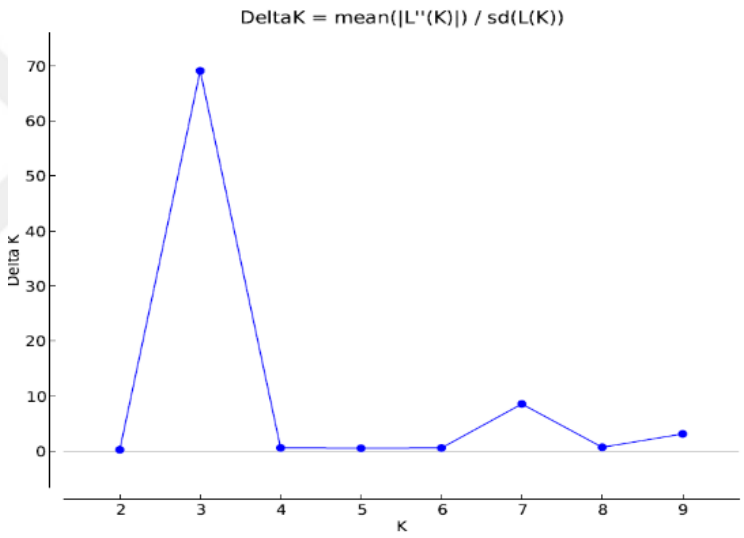


Figure 3.83. *STRUCTURE analysis for the number of populations (K = 3) of lentil accessions based on SCoT analyses.*

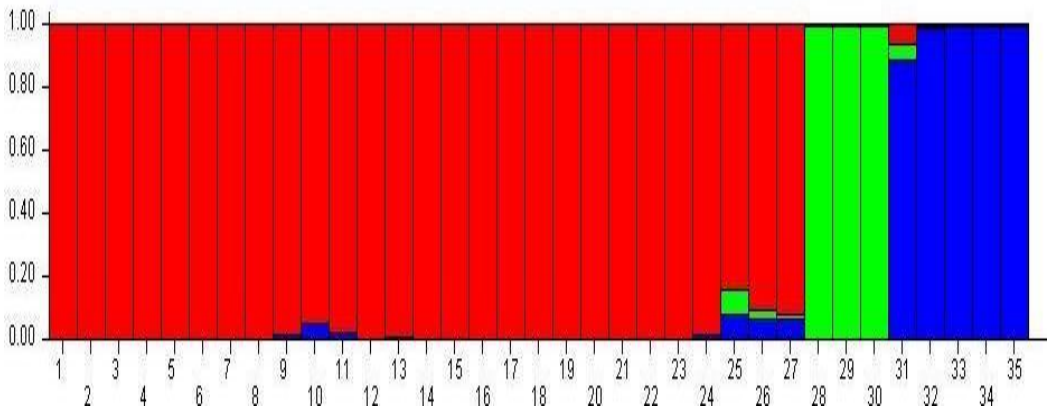


Figure 3.84. *STRUCTURE analysis of lentil accessions based on SCoT analyses.*

3.9. Data Analysis of Almond Accessions

3.9.1. Assessment of ISSR analysis

ISSR PCR results showed that 13 primers amplified a total of 200 bands from 36 almond accessions, among these bands 188 were polymorphic. The number of amplified bands varied between 10 (ISSR 817) and 20 (ISSR 834) with an average of 15 bands per primer. The band sizes ranged from 294 bp (ISSR 834) to 3667 bp (ISSR 859). The polymorphism percentage ranged from 80 to 100% with an average of 93%. In addition, the PIC values were ranged from 0.19 (ISSR 842) to 0.38 (ISSR 859) with an average of 0.25 (Table 3.22).

Table 3.23. *ISSR analysis of band patterns for almond accessions.*

Primer	Band Size (bp)	TB	MB	PB	PPB %	PIC
ISSR 807	366-2256	17	1	16	94	0.25
ISSR 808	500-1927	15	2	13	87	0.23
ISSR 809	425-1500	14	1	13	93	0.22
ISSR 810	535-2511	14	1	13	93	0.24
ISSR 814	509-2000	13	0	13	100	0.28
ISSR 815	543-1767	11	2	9	82	0.21
ISSR 817	733-2000	10	2	8	80	0.23
ISSR 829	321-1305	13	1	12	92	0.22
ISSR 834	294-2627	20	1	19	95	0.23
ISSR 842	300-1581	19	0	19	100	0.19
ISSR 847	443-2397	16	1	15	94	0.33
ISSR 851	408-1920	19	0	19	100	0.38
ISSR 859	345-3667	19	0	19	100	0.28
	SUM	200	12	188	-	-
	AVG	15	-	-	93	0.25

TB: Total number of bands, MB: Monomorphic Bands, PB: Polymorphic Bands, PPB%: Percentage of Polymorphic Bands, PIC: Polymorphism Information Content, AVG: Average.

The genetic similarity values between 36 almond accessions; 24 cultivated and 12 wild accessions were calculated based on Jaccard's similarity coefficient presented in the DendroUPGMA program. The genetically closest accessions among 36 accessions were found to be Fillis and Douma3 with a genetic similarity coefficient of 0.899. The lowest genetic similarity was found between the Wild type *A. arabica* and Ne-Plus Ultra with a genetic similarity coefficient of 0.281. Comparing the cultivated accessions together, the genetically closest accessions were between Fillis and Douma3 as indicated above, while the lowest genetic similarity was found between Oja and Douma14 with a genetic

similarity coefficient of 0.427. Comparing the wild accessions together, the genetically closest accessions was found between the wild type from Irbid/Malka and the wild type from Al-Ibrahimiye with a genetic similarity coefficient of 0.642, while the lowest genetic similarity was found between the wild type *A. arabica* and the wild type from Irbid/ Naime with a genetic similarity coefficient of 0.371 (Table 3.23).

Table 3.24. *ISSR genetic similarity matrix between almond accessions based on Jaccard similarity coefficient.*

	Ahmah	Primorski	Douma14	Chellaston	Douma3	Fillis	Ferraduel	SF121	Shamifark	Princesse
Ahmah	1	0.788	0.5	0.6	0.554	0.538	0.611	0.643	0.533	0.526
Primorski		1	0.509	0.529	0.563	0.547	0.619	0.634	0.559	0.505
Douma14			1	0.566	0.569	0.54	0.546	0.591	0.476	0.543
Chellaston				1	0.548	0.533	0.554	0.557	0.526	0.52
Douma3					1	0.899	0.636	0.635	0.648	0.554
Fillis						1	0.587	0.602	0.594	0.509
Ferraduel							1	0.729	0.587	0.628
SF121								1	0.556	0.563
Shamifark									1	0.533
Princesse										1
Ardshwar86										
A44										
Dafadii										
Texas										
Fkusii										
A155										
NePlusUltra										
Tuono										
Ai										
A13										
Douma25										
Oja										
KockTender										
Drake										
WildAjlunBaun										
WildAjlunMahna										
WildIrbidNaime										
WildAlIbrahimiye										
WildIrbidMalka										
WildAarabicas										
WildMarjAlFurs										
WildZarqa										
WildWadiShuaib										
WildAlSalt										
WildShobak										
WildDanaDistrict										

Table 3.23. (Continued) *ISSR genetic similarity matrix between almond accessions based on Jaccard similarity coefficient.*

Ardshwar86	A44	Dafadii	Texas	Fkusii	A155	NePlusUltra	Tuono	Ai	A13	Douma25
0.515	0.54	0.5	0.557	0.51	0.531	0.528	0.531	0.528	0.525	0.52
0.495	0.534	0.481	0.52	0.448	0.481	0.505	0.51	0.551	0.505	0.5
0.532	0.5	0.491	0.542	0.514	0.505	0.429	0.519	0.558	0.5	0.495
0.524	0.596	0.481	0.615	0.535	0.495	0.538	0.54	0.523	0.549	0.561
0.528	0.523	0.5	0.524	0.495	0.544	0.45	0.544	0.527	0.552	0.519
0.5	0.495	0.486	0.538	0.495	0.543	0.451	0.543	0.5	0.509	0.491
0.505	0.622	0.549	0.592	0.53	0.615	0.516	0.566	0.546	0.514	0.621
0.551	0.606	0.566	0.547	0.491	0.567	0.505	0.567	0.62	0.546	0.573
0.505	0.564	0.553	0.582	0.5	0.49	0.554	0.521	0.462	0.515	0.495
0.577	0.75	0.515	0.525	0.574	0.765	0.581	0.515	0.486	0.481	0.733
1	0.544	0.519	0.529	0.53	0.49	0.469	0.449	0.532	0.544	0.51
	1	0.559	0.539	0.604	0.696	0.651	0.56	0.527	0.495	0.782
		1	0.5	0.43	0.505	0.439	0.49	0.518	0.514	0.495
			1	0.659	0.561	0.527	0.561	0.514	0.539	0.551
				1	0.613	0.495	0.546	0.473	0.467	0.568
					1	0.605	0.551	0.464	0.472	0.756
						1	0.551	0.485	0.434	0.671
							1	0.505	0.56	0.525
								1	0.527	0.538
									1	0.52
										1

Oja	KockTender	Drake	WildAjlunBaun	WildAjlunMahna	WildIrbidNaime	WildAlIbrahimiye
0.529	0.598	0.685	0.449	0.525	0.51	0.444
0.539	0.557	0.621	0.431	0.462	0.462	0.455
0.427	0.477	0.464	0.431	0.46	0.447	0.524
0.489	0.51	0.54	0.475	0.52	0.422	0.47
0.495	0.592	0.559	0.425	0.481	0.455	0.476
0.526	0.544	0.528	0.426	0.482	0.43	0.476
0.533	0.551	0.535	0.455	0.515	0.515	0.48
0.505	0.538	0.567	0.449	0.519	0.505	0.486
0.556	0.522	0.49	0.392	0.426	0.412	0.447
0.478	0.652	0.5	0.42	0.466	0.525	0.459
0.468	0.551	0.582	0.441	0.486	0.444	0.495
0.511	0.577	0.545	0.423	0.495	0.495	0.447
0.484	0.49	0.476	0.4	0.486	0.458	0.48
0.462	0.562	0.5	0.51	0.54	0.525	0.475
0.446	0.564	0.515	0.434	0.525	0.495	0.444
0.452	0.62	0.49	0.469	0.545	0.561	0.436
0.512	0.534	0.484	0.413	0.448	0.463	0.394
0.534	0.552	0.476	0.44	0.515	0.417	0.465
0.455	0.548	0.562	0.418	0.46	0.447	0.427
0.479	0.53	0.545	0.451	0.495	0.467	0.433
0.441	0.591	0.557	0.474	0.535	0.551	0.426
1	0.517	0.436	0.38	0.478	0.36	0.438
	1	0.656	0.41	0.531	0.5	0.479
		1	0.44	0.53	0.515	0.48
			1	0.543	0.559	0.539
				1	0.556	0.537
					1	0.49
						1

Table 3.23. (Continued) *ISSR genetic similarity matrix between almond accessions based on Jaccard similarity coefficient.*

WildIrbidMalka	WildAarabicas	WildMarjAlFurs	WildZarqa	WildWadiShuaib	WildAlSalt	WildShobak	WildDanaDistrict
0.519	0.336	0.471	0.505	0.48	0.433	0.5	0.449
0.5	0.379	0.481	0.531	0.505	0.443	0.467	0.431
0.509	0.361	0.491	0.495	0.5	0.43	0.478	0.405
0.5	0.336	0.427	0.415	0.49	0.443	0.454	0.431
0.491	0.364	0.446	0.462	0.495	0.436	0.5	0.398
0.466	0.366	0.46	0.463	0.481	0.438	0.486	0.387
0.509	0.344	0.477	0.495	0.545	0.426	0.463	0.427
0.526	0.385	0.495	0.529	0.519	0.434	0.495	0.476
0.453	0.311	0.417	0.433	0.484	0.406	0.446	0.378
0.45	0.358	0.417	0.475	0.48	0.38	0.457	0.464
0.523	0.365	0.423	0.495	0.5	0.426	0.505	0.455
0.478	0.341	0.42	0.394	0.481	0.384	0.42	0.383
0.509	0.376	0.436	0.438	0.471	0.426	0.463	0.413
0.505	0.318	0.431	0.49	0.452	0.407	0.431	0.408
0.438	0.325	0.43	0.417	0.451	0.419	0.43	0.449
0.43	0.331	0.409	0.465	0.442	0.373	0.449	0.426
0.38	0.281	0.41	0.411	0.374	0.343	0.396	0.34
0.43	0.331	0.422	0.451	0.442	0.398	0.409	0.398
0.471	0.361	0.427	0.416	0.446	0.405	0.427	0.393
0.546	0.384	0.445	0.505	0.481	0.409	0.459	0.396
0.446	0.333	0.439	0.455	0.475	0.389	0.439	0.388
0.417	0.299	0.484	0.456	0.511	0.396	0.394	0.396
0.455	0.307	0.49	0.51	0.47	0.451	0.476	0.439
0.538	0.374	0.49	0.495	0.546	0.51	0.505	0.469
0.535	0.4	0.515	0.505	0.527	0.459	0.441	0.432
0.519	0.393	0.56	0.536	0.589	0.505	0.515	0.48
0.562	0.371	0.486	0.475	0.48	0.462	0.515	0.422
0.642	0.385	0.558	0.549	0.554	0.548	0.51	0.457
1	0.44	0.537	0.514	0.563	0.5	0.537	0.449
	1	0.421	0.398	0.44	0.474	0.398	0.388
		1	0.624	0.628	0.588	0.491	0.427
			1	0.587	0.564	0.557	0.458
				1	0.568	0.561	0.527
					1	0.571	0.49
						1	0.581
							1

An UPGMA dendrogram showing the genetic relationships between the almond accessions were obtained using the MEGA 11 software. The UPGMA clustering pattern from ISSR analyses grouped the 36 almond accessions into three clusters: cluster I included the wild type *A. arabica*, cluster II included all the wild accessions together separated into smaller groups, and cluster III included all the cultivated accessions together separated into smaller groups (Fig. 3.85).

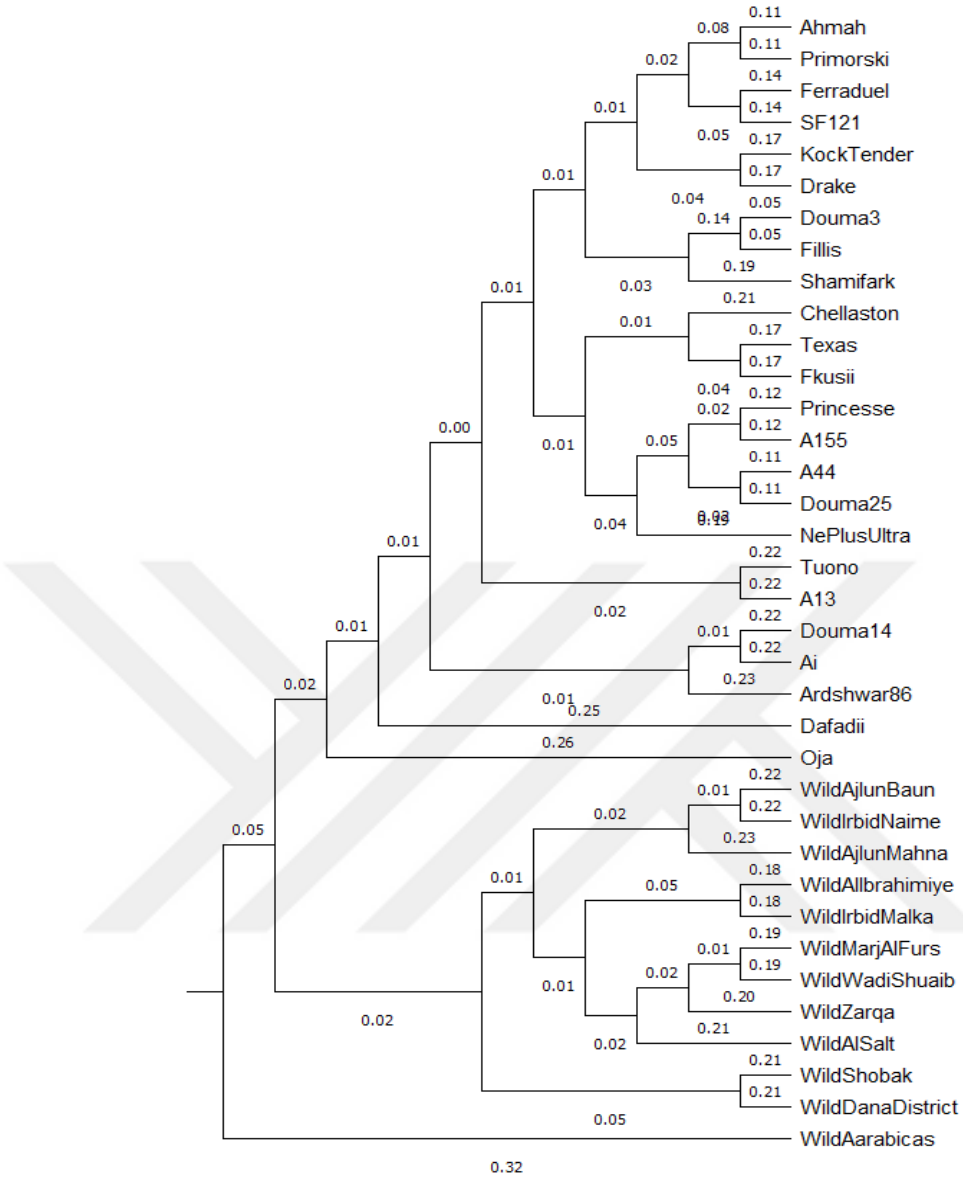


Figure 3.85. *UPGMA clustering pattern of 36 almond accessions based on the genetic similarity values of ISSR analyses.*

The genetic structure of the 36 almond accessions was determined using the STRUCTURE software clustering model. The software assumed a model of admixture degree for each individual plant and the populations (K) that contribute to the genotype of each individual. The analysis was performed with K = 2-10, each with 10 iterations (Table 3.24). The results showed the highest peak at K= 3 indicating the presence of three major clusters (Fig. 3.86 and Fig. 3.87).

Table 3.25. *Average mean values of K=2-10 for almond accessions based on ISSR analyses.*

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
2	10	-2762.850000	1.497591	—	—	—
3	10	-2779.710000	106.026134	-16.860000	846.000000	7.979165
4	10	-3642.570000	844.773225	-862.860000	1181.170000	1.398210
5	10	-3324.260000	1125.075792	318.310000	403.580000	0.358714
6	10	-3409.530000	719.727753	-85.270000	3261.030000	4.530922
7	10	-6755.830000	7942.144570	-3346.300000	4778.450000	0.601657
8	10	-5323.680000	4994.553264	1432.150000	613.430000	0.122820
9	10	-3278.100000	601.707138	2045.580000	1905.090000	3.166142
10	10	-3137.610000	166.271100	140.490000	—	—

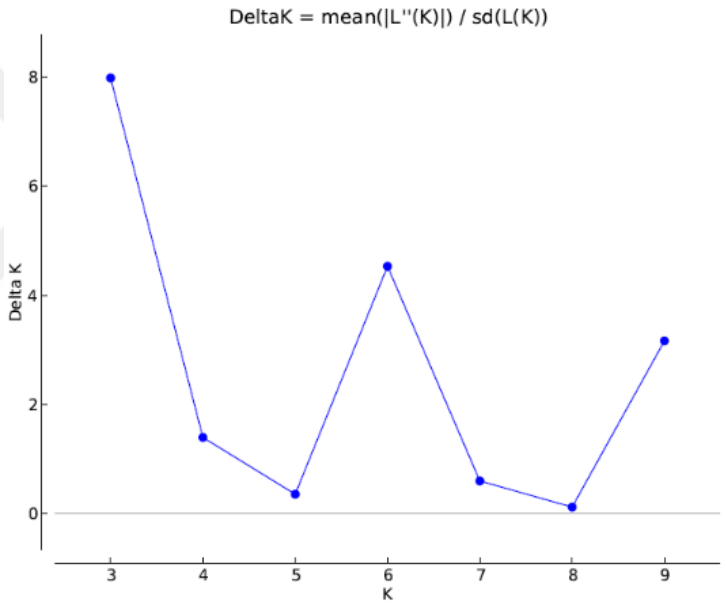


Figure 3.86. *STRUCTURE analysis for the number of populations (K = 3) of almond accessions based on ISSR analyses.*

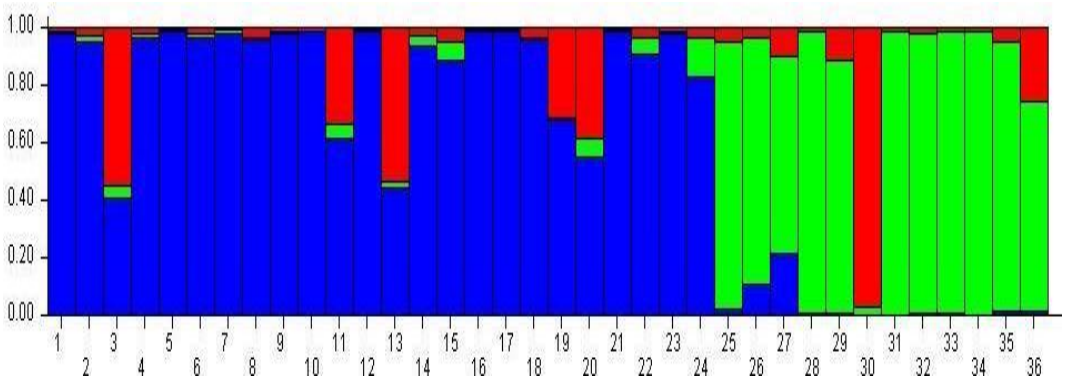


Figure 3.87. *STRUCTURE analysis of almond accessions based on ISSR analyses.*

3.9.2. Assessment of SCoT Analysis

SCoT PCR results showed that 10 primers amplified a total of 193 bands from 36 almond accessions. The number of bands varied between 13 (SCoT 28) and 23 (SCoT 12) with an average of 19.3 bands per primer. Among 193 bands 174 were polymorphic, the polymorphism percentage ranged from 84 to 95% with an average of 90%. The band sizes ranged from 240 bp (SCoT 21) to 3681 bp (SCoT 16). In addition, PIC values ranged from 0.19 (SCoT 28) to 0.31 (SCoT 34) with an average of 0.25 (Table 3.25).

Table 3.26. *SCoT analysis of band patterns for almond accessions.*

Primer	Band Size (bp)	TB	MB	PB	PPB %	PIC
SCoT 12	313-2813	23	2	21	91	0.3
SCoT 15	282-2630	22	3	19	86	0.24
SCoT 16	687-3681	14	1	13	93	0.27
SCoT 17	242-2548	18	1	17	94	0.22
SCoT 18	323-2823	22	3	19	86	0.25
SCoT 19	482-3000	23	2	21	91	0.28
SCoT 21	240-2791	19	3	16	84	0.22
SCoT 28	452-2974	13	2	11	85	0.19
SCoT 32	400-3428	21	1	20	95	0.23
SCoT 34	419-3187	18	1	17	94	0.31
	SUM	193	19	174	-	-
	AVG	19.3	-	-	90	0.25

TB: Total number of bands, MB: Monomorphic Bands, PB: Polymorphic Bands, PPB%: Percentage of Polymorphic Bands, PIC: Polymorphism Information Content, AVG: Average.

The genetic similarity matrix was produced between 36 almond accessions (24 cultivated and 12 wild) based on Jaccard's similarity coefficient. The genetically closest accessions were Fillis and Douma3 with a genetic similarity coefficient of 0.818. The lowest genetic similarity was found between A13 and the wild type *A. arabica* with a genetic similarity coefficient of 0.289. Comparing the cultivated accessions together, the genetically closest accessions were Fillis and Douma3 as indicated above, while the lowest genetic similarity was found between A13 and Primorski with a genetic similarity coefficient of 0.421. Comparing the wild types together, the genetically closest types were found in the wild type from Dana District and the wild type from Shobak with a genetic similarity coefficient of 0.789, while the lowest genetic similarity was found between the wild type *A. arabica* and the wild type Ajlun/Mahna, and between the wild type *A.*

arabica and the wild type from Al-Salt with the same genetic similarity coefficient of 0.455 (Table 3.26).

Table 3.27. *SCoT Genetic similarity matrix between Almond accessions based on Jaccard similarity coefficient.*

	Ahmah	Primorski	Douma14	Chellaston	Douma3	Fillis	Ferraduel	SF121	Shamifark	Princesse
Ahmah	1	0.559	0.535	0.535	0.568	0.581	0.485	0.464	0.589	0.596
Primorski		1	0.516	0.532	0.516	0.545	0.463	0.44	0.5	0.511
Douma14			1	0.624	0.543	0.505	0.604	0.438	0.544	0.587
Chellaston				1	0.558	0.553	0.62	0.533	0.527	0.656
Douma3					1	0.818	0.625	0.517	0.563	0.644
Fillis						1	0.621	0.547	0.576	0.64
Ferraduel							1	0.581	0.539	0.6
SF121								1	0.536	0.545
Shamifark									1	0.575
Princesse										1
Ardshwar86										
A44										
Dafadii										
Texas										
Fkusii										
A155										
NePlusUltra										
Tuono										
Ai										
A13										
Douma25										
Oja										
KockTender										
Drake										
WildAjlunBaun										
WildAjlunMahna										
WildIrbidNaime										
WildAlIbrahimiye										
WildIrbidMalka										
WildAarabicas										
WildMarjAlFurs										
WildZarqa										
WildWadiShuaib										
WildAlSalt										
WildShobak										
WildDanaDistrict										

Table 3.26. (continued) *SCoT Genetic similarity matrix between almond accessions based on Jaccard similarity coefficient.*

Ardshwar86	A44	Dafadii	Texas	Fkusii	A155	NePlusUltra	Tuono	Ai	A13	Douma25	Oja
0.541	0.63	0.573	0.463	0.521	0.531	0.609	0.588	0.568	0.475	0.449	0.577
0.556	0.527	0.474	0.485	0.452	0.527	0.489	0.459	0.453	0.421	0.472	0.511
0.564	0.57	0.581	0.495	0.56	0.505	0.516	0.579	0.48	0.495	0.484	0.552
0.562	0.637	0.63	0.524	0.559	0.536	0.547	0.594	0.558	0.559	0.567	0.567
0.582	0.644	0.548	0.51	0.598	0.571	0.584	0.615	0.511	0.616	0.517	0.64
0.527	0.699	0.596	0.461	0.575	0.621	0.616	0.576	0.522	0.612	0.529	0.6
0.629	0.694	0.667	0.505	0.647	0.548	0.543	0.591	0.607	0.556	0.495	0.581
0.505	0.679	0.489	0.516	0.535	0.495	0.523	0.505	0.57	0.5	0.542	0.527
0.484	0.612	0.551	0.495	0.511	0.505	0.667	0.533	0.462	0.462	0.5	0.628
0.667	0.778	0.648	0.505	0.667	0.694	0.614	0.626	0.663	0.573	0.619	0.598
1	0.686	0.604	0.53	0.602	0.559	0.538	0.52	0.655	0.533	0.505	0.526
	1	0.686	0.535	0.647	0.674	0.632	0.574	0.663	0.591	0.6	0.633
		1	0.53	0.64	0.576	0.554	0.637	0.6	0.584	0.505	0.542
			1	0.574	0.52	0.515	0.545	0.495	0.451	0.532	0.566
				1	0.591	0.551	0.6	0.635	0.545	0.535	0.554
					1	0.596	0.558	0.625	0.591	0.639	0.598
						1	0.57	0.549	0.453	0.558	0.648
							1	0.564	0.6	0.522	0.589
								1	0.562	0.5	0.49
									1	0.571	0.538
										1	0.598
											1

KockTender	Drake	WildAjlunBaun	WildAjlunMahna	WildIrbidNaime	WildAlIbrahimiye	WildIrbidMalka
0.564	0.49	0.505	0.505	0.574	0.598	0.49
0.511	0.532	0.452	0.435	0.451	0.44	0.436
0.554	0.525	0.495	0.479	0.49	0.525	0.511
0.521	0.54	0.51	0.511	0.549	0.525	0.526
0.538	0.526	0.448	0.447	0.476	0.48	0.463
0.551	0.49	0.473	0.457	0.471	0.49	0.442
0.516	0.552	0.458	0.473	0.471	0.419	0.474
0.565	0.5	0.435	0.518	0.45	0.439	0.553
0.595	0.479	0.462	0.477	0.505	0.495	0.462
0.533	0.505	0.522	0.505	0.577	0.62	0.573
0.464	0.5	0.533	0.484	0.51	0.546	0.567
0.621	0.49	0.538	0.557	0.561	0.568	0.591
0.527	0.456	0.516	0.533	0.525	0.515	0.469
0.52	0.57	0.423	0.45	0.519	0.467	0.542
0.489	0.526	0.432	0.446	0.461	0.465	0.495
0.5	0.52	0.458	0.489	0.5	0.568	0.505
0.58	0.581	0.484	0.5	0.541	0.531	0.5
0.559	0.545	0.5	0.5	0.481	0.545	0.469
0.474	0.542	0.527	0.478	0.52	0.542	0.562
0.457	0.48	0.447	0.478	0.461	0.48	0.447
0.565	0.516	0.435	0.5	0.51	0.484	0.535
0.6	0.567	0.416	0.458	0.5	0.476	0.444
1	0.521	0.505	0.506	0.562	0.505	0.522
	1	0.408	0.42	0.463	0.426	0.465
		1	0.622	0.637	0.648	0.581
			1	0.64	0.632	0.602
				1	0.699	0.637
					1	0.648
						1

Table 3.26. (continued) *SCoT Genetic similarity matrix between almond accessions based on Jaccard similarity coefficient.*

WildAarabicas	WildMarjAlFurs	WildZarqa	WildWadiShuaib	WildAlSalt	WildShobak	WildDanaDistrict
0.377	0.49	0.434	0.515	0.475	0.516	0.511
0.315	0.396	0.439	0.447	0.421	0.462	0.438
0.378	0.465	0.437	0.474	0.464	0.538	0.5
0.381	0.51	0.452	0.521	0.526	0.521	0.533
0.316	0.436	0.408	0.458	0.463	0.442	0.483
0.333	0.416	0.43	0.453	0.473	0.467	0.494
0.373	0.475	0.446	0.469	0.489	0.5	0.478
0.349	0.484	0.453	0.494	0.517	0.494	0.488
0.333	0.448	0.376	0.457	0.478	0.489	0.482
0.411	0.587	0.537	0.602	0.573	0.551	0.529
0.407	0.547	0.564	0.56	0.484	0.527	0.54
0.411	0.553	0.521	0.602	0.556	0.568	0.565
0.394	0.515	0.47	0.527	0.516	0.562	0.523
0.383	0.54	0.481	0.461	0.451	0.49	0.396
0.374	0.511	0.464	0.473	0.478	0.506	0.466
0.373	0.587	0.537	0.516	0.522	0.533	0.478
0.38	0.469	0.44	0.511	0.484	0.511	0.489
0.396	0.515	0.485	0.526	0.516	0.56	0.505
0.402	0.611	0.559	0.538	0.495	0.505	0.5
0.289	0.511	0.464	0.489	0.478	0.457	0.466
0.336	0.516	0.516	0.547	0.553	0.494	0.471
0.339	0.433	0.406	0.485	0.505	0.516	0.447
0.383	0.474	0.444	0.533	0.505	0.552	0.529
0.405	0.452	0.466	0.446	0.45	0.43	0.423
0.47	0.511	0.511	0.539	0.545	0.654	0.613
0.455	0.598	0.562	0.614	0.622	0.617	0.595
0.495	0.582	0.566	0.63	0.656	0.652	0.578
0.515	0.659	0.573	0.604	0.611	0.625	0.586
0.5	0.632	0.596	0.651	0.581	0.595	0.593
1	0.515	0.485	0.465	0.455	0.48	0.458
	1	0.721	0.607	0.596	0.556	0.517
		1	0.625	0.632	0.573	0.517
			1	0.734	0.646	0.625
				1	0.675	0.633
					1	0.789
						1

An UPGMA dendrogram showing the genetic relationships between the almond accessions was obtained using the MEGA 11 software. The UPGMA clustering pattern from SCoT analyses grouped the 36 almond accessions into three clusters: cluster I included only the wild type *A. arabica*, cluster II included all the rest wild accessions together separated into smaller groups, and cluster III included all the cultivated accessions together separated into smaller groups (Fig. 3.88).

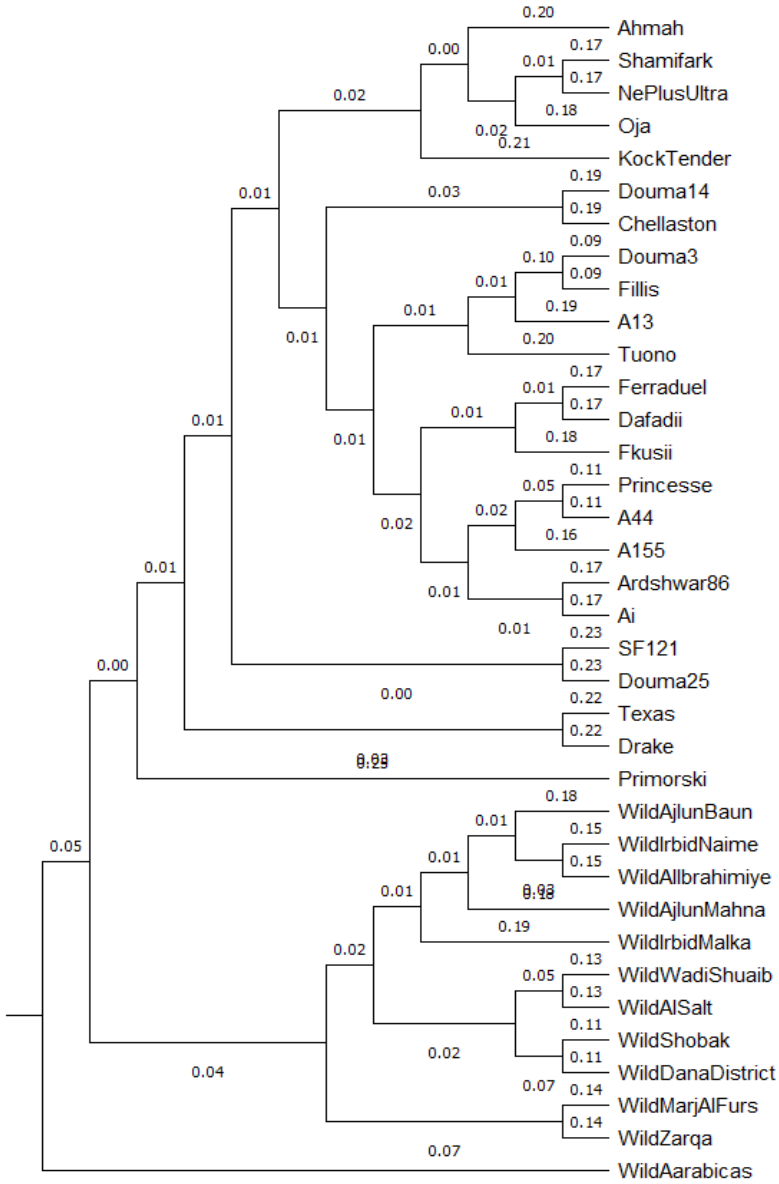


Figure 3.88. *UPGMA clustering pattern of 36 almond accessions based on the genetic similarity of SCoT analyses.*

The genetic structure of the 36 almond accessions was performed using the STRUCTURE software clustering model. The software assumed a model of admixture degree for each individual plant and the populations (K) that contribute to the genotype of each individual. The analysis was performed with K = 1-9, each with 10 iterations (Table 3.27). The results showed the highest peak at K= 2 indicating the presence of two major clusters (Fig. 3.89 and Fig. 3.90).

Table 3.28. *Average mean values of K=1-9 for almond accessions based on SCoT analyses.*

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-2537.730000	0.567744	—	—	—
2	10	-2284.170000	9.481333	253.560000	288.910000	30.471455
3	10	-2319.520000	28.638312	-35.350000	39.960000	1.395334
4	10	-2314.910000	109.113289	4.610000	149.430000	1.369494
5	10	-2459.730000	291.561939	-144.820000	188.850000	0.647718
6	10	-2415.700000	157.137823	44.030000	190.620000	1.213075
7	10	-2562.290000	330.513037	-146.590000	89.810000	0.271729
8	10	-2619.070000	192.562492	-56.780000	3235.660000	16.803168
9	10	-5911.510000	9723.999117	-3292.440000	—	—

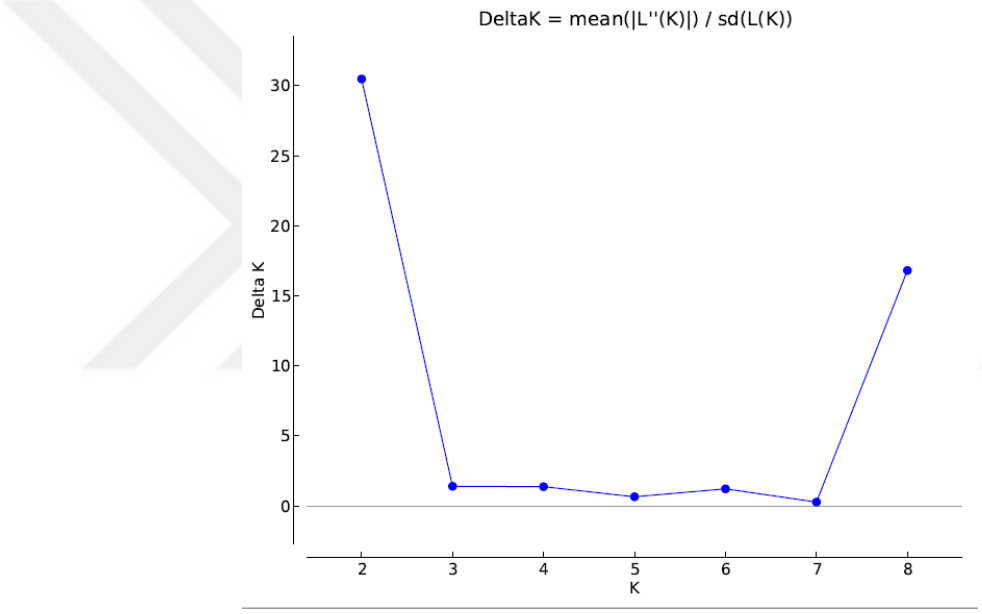


Figure 3.89. *STRUCTURE analysis for the number of populations (K = 2) of almond accessions based on SCoT analyses.*

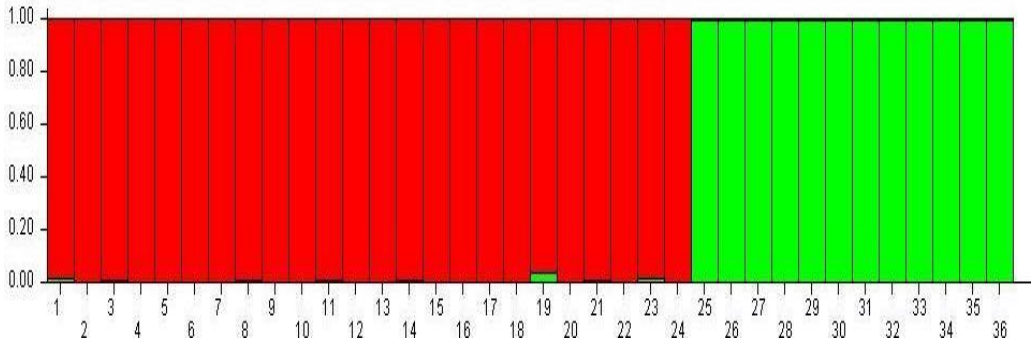


Figure 3.90. *STRUCTURE analysis of almond accessions based on SCoT analyses.*

4. DISCUSSION

Plant genetic resources is a key component of the global genetic biodiversity, which are essential for humanity, food security and livelihoods. Landraces are important genetic resources that improve gene pools of modern cultivars through introducing new alleles with its specific genetic background which can be used in genetic research programs (Pakseresht et al., 2013). The genetic diversity is the key factor for population survival and evolution (González, Gómez-Silva, Ramírez, & Fontúrbel, 2020) and it is important for crop improvement, efficient management, and conservation of germplasm resources (Tahir & Karim, 2011), in addition to maintaining ecosystem functions, stability, and has the same impacts on community structure and ecosystem processes as species diversity (Hoban, et al., 2020).

Genebanks were created in the middle of the twentieth century to preserve cultivated biodiversity when landraces began to be replaced by modern varieties and was accepted as a necessity to safeguard the future (Díez, et al., 2018). Many accessions per crop can be stored in the plant genebank. There is always a limit to the number of accessions that can be conserved and utilized, so that those large numbers of genebank collections have some challenges such as unnecessary duplicates within and between genebanks. Different types of molecular markers have been used to estimate the genetic diversity of conserved accessions in the genebanks. However, DNA molecular markers allow unlimited numbers of polymorphic marker loci evaluation (Nguyen, Taylor, Redden, & Ford, 2004).

The appearance of different molecular techniques led breeders to assess genetic diversity based on data generated by different molecular markers, which provided a means of rapid analysis of germplasm and estimates of genetic diversity, which were often found to confirm phenotypic data (Rauf, da Silva, Khan, & Naveed, 2010). Molecular markers have succeeded in identification among accessions, clarifying synonyms, characterizing mislabeled cultivars, establishment of genetic similarities or geographical origins and giving hints about the process of domestication (La, 2009). The genetic diversity between the various landraces remains to be studied and molecular markers are greatly useful in quantifying this diversity (Tahir & Karim, 2011).

The aims of this study were to characterize the genetic diversity levels within chickpea, lentil and almond genotypes growing in different regions in Jordan, to identify their DNA banding patterns and check possible duplications of accessions in the seed

genebank and investigate the relative genetic contribution between local cultivated landraces and some of its local wild landraces in the case of lentils and almonds using ISSR and SCoT markers.

Jordan is considered a center of origin of wheat, barley, food, and forage legumes and many fruit trees such as almond and figs. A significant diversity can be found in the country among cultivated landraces of grapes, olives, wheat, barley, chickpea, and lentils (Al-Fyad, et al., 2007). The country has established a genetic resources unit in NARC from 1993 (Syouf & Duwayri, 1996), recently NARC's seed genebank, hosts more than 4,000 seed accessions of different plant groups, including crop and landraces. Genetic diversity studies had not been used on most of the accessions, which this is a need and important step to differentiate the accessions genetically avoiding many problems as duplication which is a main subject to discuss.

The collection of chickpea accessions located in the seed genebank contains 20 accessions collected from 10 different regions of Jordan (Table 2.1). In this study, we used 18 of them due to germination problem of 2 accessions. The number of lentil accessions presented in the seed genebank is 97, 75 are accessions of *L. culinaris* cultivated accessions, 22 are the wild landrace of *L. ervoides*. In this study 27 *L. culinaris* accessions and 8 wild landraces of *L. ervoides* were used. For almond, 24 cultivated accessions were adopted from Al-Mushaqar Station field of NARC, in addition 12 wild landraces were collected from different regions in Jordan.

4.1. Genetic Diversity of Chickpea Accessions

In order to estimate genetic diversity among 18 Jordanian chickpea accessions 13 ISSR and 10 SCoT primers were used. ISSR analyses produced 135 bands of which 110 were polymorphic, with an average of 72 % polymorphism. The number of amplified bands varied between 2 (ISSR 817) and 18 (ISSR 811) with an average of 10.4 bands per primer. The average PIC value of used ISSR primers was calculated as 0.17. On the other hand, 10 SCoT primers amplified reproducible 166 bands, among these 129 were polymorphic (PPB: 75%). The number of amplified bands varied between 8 (SCoT 3) and 25 (SCoT 32) with an average of 16.6 bands per primer. The average PIC value of SCoT primers was calculated as 0.26. As seen from these results the number of bands and polymorphism levels of SCoT primers were higher than ISSR. In addition, the average PIC value of SCoT primers (0.26) was also higher than that of ISSR (0.17). Although polymorphism rate for these two primers were high, their informativeness (PIC) was not

since the highest PIC value for a dominant marker is said to be 0.5 (De Riek, Calsyn, Everaert, Van Bockstaele, & De Loose, 2001). These results showed that SCoT primers were more efficient and informative than ISSR to estimate genetic diversity in studied Jordanian chickpea accessions.

Both ISSR and SCoT primers used in our study generated higher polymorphism levels than that of some previous studies. Chickpea has known with a narrow genetic base and minimal polymorphism as shown previously by molecular markers such as seed protein, isozyme and RFLP analysis (Iruela, Rubio, Cubero, Gil, & Millan, 2002; Sani, et al., 2018). Using RAPD and ISSR markers, a molecular analysis was carried out to determine the genetic diversity and relationships among chickpea cultivars, 33 bands overall were produced by the 5 polymorphic primers with 63.6 % polymorphism (Tahir and Karim, 2011). In another study, 10 SCoT primers used to fingerprint 10 genotypes of *Cicer arietinum*, 33 polymorphic bands out of 94 amplified bands were recorded and the polymorphism percentage was 35 % (Serag, 2021).

On the other hand, some studies obtained higher polymorphism levels than our studies. For example, ISSR and SCoT markers were used for the analysis of genetic relationships among 38 accessions of 8 annual *Cicer* species including the cultivated *C. arietinum*, which detected a high level of polymorphism about 97 % (Amirmoradi, Talebi, & Karami, 2012). Results obtained from the same study confirmed that *C. arietinum* has a narrowest genetic base among all the annual species and the wild *C. reticulatum* genetically is the closest species to C. *arietinum*. Aggarwal et al., (2015) reported that 26 ISSR primers yielded a total of 232 bands representing the genetic diversity of 125 cultivars of chickpea with Indian origin, 213 of which were polymorphic (91.8 %) with an average of 9 bands per primer. The polymorphic information content was 0.72. In addition, Hajibarat et. al. (2015) showed that 9 SCoT primers amplified a total of 145 bands from 48 Iranian chickpea accessions, among these bands 133 were polymorphic. The average PIC value was 0.45 and showed a high level of polymorphism and diversity. These three studies obtained higher polymorphism levels than our study, because they used different *Cicer* species and high number of accessions. However, Pakseresht et al. (2013) studied 40 Iranian chickpea genotypes using 7 ISSR and 10 SCoT primers. The average PIC values were 0.216 for ISSR markers and 0.232 for the SCoT markers that was close to the results of our study, and they concluded similarly that SCoT marker was more informative than ISSR markers.

The genetic similarity matrix between chickpea accessions was calculated based on Jaccard's similarity coefficient. For ISSR markers, the genetic similarity values of Jordanian chickpea were moderate, between 0.769 and 0.452. The most genetically closest accessions were 4537 and 4361 from Maan (0.769) and the most distant accessions were 2798 from Madaba and 2795 from Zarqa (0.452). While for SCoT markers, the genetic similarity values were between 0.744 and 0.468, the most genetically closest accessions were 2779 from Mafraq and 2777 from Balqa (0.744), and genetically the lowest accessions were 3590 from Unknown and 2779 from Mafraq (0.468). The similarity coefficient values for the two marker systems were almost the same, but they did not produce similar clusters in the UPGMA dendrograms. The difference between the two markers is due to the different DNA sequence presented in each primer, which tracked different readings among the PCR analyses, producing different band profiles.

According to the clustering pattern obtained from ISSR analyses, the 18 chickpea accessions were grouped into 2 main clusters: cluster I included only one variety 2795 from Zarqa, and cluster II divided into two sub clusters, the first sub cluster included 2801 from unknown location and the second sub cluster included all the chickpea genotypes divided into smaller groups. Group I included 2775, 2780 from Amman, 2779 from Mafraq and 2777 from Balqa were the closest together, group II included, 2788, 3588 from unknown locations, 2787, 2796, 4361, 4537, 2793 from Maan and 2798 from Madaba, and group III included 2791 from Jerash, 2794 from Karak and 2799 from Irbid together. Because 2801, 3590, 2788 and 3588 were from unknown locations we cannot know from which place or city they originated but the last two unknown accessions are genetically close to Maan accessions as seen in figure 3.73.

On the other hand, the dendrogram produced using SCoT analyses grouped the 18 chickpea accessions into 2 main clusters: cluster I was included 3590 and 3588 from unknown locations, cluster II was divided into smaller two groups, Group I included 2801 from unknown location, 2799 from Irbid, 2798 from Madaba; 2795 from Zarqa; 2794 from Karak; 2788 from unknown location, and 2791 from Jerash, while Group II included 2793, 4537, 4361, 2796 and 2787 from Maan, while cluster III was included 2779 from Mafraq, 2777 from Balqa, 2780 and 2775 from Amman. Here 3590 from unknown location was also placed in separate cluster but with other unknown location accession (3588), and 2801 from unknown location was genetically close to them, while the unknown (27880) was placed into another group. In SCoT analyses 2795 from Zarqa was

genetically close to accession 2798 from Madaba and 2794 from Karak, but in ISSR analyses 2795 from Zarqa was separated in different cluster. It can be said that in both ISSR, and SCoT analyses the accessions from Maan were either grouped in the same cluster or located close to each other which mean that these accessions are genetically close.

The ISSR genetic structure of 18 Jordanian chickpea accessions were grouped by the STRUCTURE software clustering model into 3 clusters K=3 with three different colors green, red, and blue. These three clusters were presented in all the accessions, the red cluster presented somehow in equal balance. However, the green and blue clusters equaled in balance between the following accessions: 2780 from Amman, 2777 from Balqa, 2791 from Jerash, and 2788 from unknown location. The blue cluster presented more in 2793, 4537, 4361, 2796, 2787 from Maan, 3588 from unknow location and 2798 from Madaba. While the green cluster presented more in 2775 from Amman, 2779 from Mafraq, 2795 from Zarqa, 2794 from Karak, 2799 from Irbid, 2801, and 3590 from unknown locations as shown in figure 3.75.

Also, the SCoT genetic structure grouped the 18 Jordanian chickpea accessions into 3 clusters K=3 with three different colors green, red and blue but in different way. This time the arrangement of the three clusters were as the following: the cluster with the green color was the most in 2775 and 2780 from Amman, 2779 from Mafraq and 2777 from Balqa and 2796 from Maan. The red and blue clusters ranged in balance between 2787, 2793, 4537 and 4361 from Maan, 2791 from Jerash, 2795 from Zarqa, 2794 from Karak, 2798 from Madaba, 2799 from Irbid, 2801, 3590 and 2788 from unknown locations. While the cluster with the red color was the most only in 3588 from unknown location as shown in figure 3.78. SCoT marker proved to be more informative distinguishing closely related genotypes than ISSR marker.

These findings of STRUCTURE analyses implied that the 3 clusters or populations were mixed together over the years as they had been cultivated throughout the country. Jordan has four distinct biogeographical regions (Fig. 4.1), whilst the cultivated areas for chickpeas mainly found in the Mediterranean and Irano-Turanian regions. In this study we cannot proved any differences between the cultivated accessions according to the biogeographical areas via ISSR analyses, however SCoT PCR analysis results showed that 2775 and 2780 from Amman, 2779 from Mafraq and 2777 from Balqa were the most genetically different accessions from the rest. However, the SCoT marker had been more

informative than ISSR marker as presented earlier. Amman, Mafraq and Balqa provinces presented in the Middle to North of Jordan and considered near to each other with almost the same biogeographical regions mixed between the Mediterranean and the Irano-Turanian.

As described earlier, all of the 18 chickpea accessions are genetically related to each other in some ways, even that they were geographically distant landraces but genetically similar. There is an explanation for this similarity that the landraces were moved by farmers from one site to another which were selected for their specific adaptation to local environmental conditions. Another explanation can be also mentioned that they originated from the same ancestor. All of chickpea accessions stored in NARC were from the kabuli type. It can be seen from their seed external features such as seed shape and size. The accessions from Amman, Mafraq and Balqa provinces, have almost the same genetic structure, in addition to the accessions from Maan, they were all clustered in the same group and were genetically almost the same. According to our PCR results there is no duplication among the stored accessions.

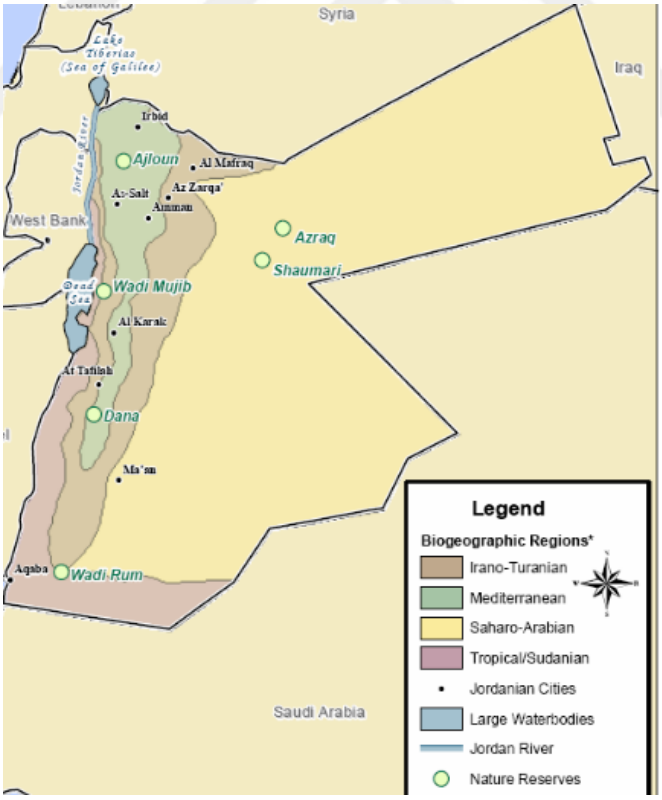


Figure 4.1. *The four distinct biogeographical regions of Jordan.*

4.2. Genetic Diversity of Lentil Accessions

To estimate genetic diversity among 35 Jordanian lentil accessions 13 ISSR and 10 SCoT primers were used. The 13 ISSR primers generated a total of 170 bands, out of which 157 were polymorphic (PPB: 92 %). The number of bands varied from 8 (ISSR 813) to 20 (ISSR 808), with an average of 13 bands per primer. The average PIC value of ISSR primers was calculated as 0.24. On the other hand, the 10 SCoT primers amplified reproducible 174 bands, 155 were polymorphic, with an average of 89 % polymorphism. The number of bands were between 13 (SCoT 21) and 26 (SCoT 18), with an average of 17.4 bands per primer. The average PIC value of SCoT primers was 0.27. Comparing the SCoT and ISSR total band numbers, it was higher in SCoT primers. In addition to the average PIC value of SCoT primers (0.27) was also higher than ISSR (0.24). While the polymorphism level was higher in ISSR primers (92 %) than SCoT primers (89%).

In a previous study, 46 accessions of lentil, 31 of them were originated in Italy, 6 ISSR primers were utilized, produced a total number of 74 bands, revealed a low degree of useful polymorphism (65 %) (Sonnante & Pignone, 2001). ISSR marker proved to be more useful for distinguishing closely related genotypes from RAPD markers (Sonnante and Pignone, 2001; Laghetti et. al., 2008). Toklu, et al. (2009) reported that 38 lentil landraces with 6 commercial cultivars from southeast Turkey were molecularly characterized using 14 ISSR primers, which generated a total of 125 bands, out of them 105 were polymorphic (84.9 %), and the average of bands per primer were 8.9. While the PIC value calculated as 0.677. El-Nahas et. al. (2011) reported that 5 ISSR primers were used to study the drought tolerance of 6 lentil genotypes, producing a total of 65 bands and an average of 46% polymorphism. Another study was carried out to identify the genetic diversity of 6 lentil cultivars, 17 SCoT primers were amplified 84 bands, 57 bands were polymorphic (67.86 %), the size of amplified bands ranged between 211 and 2763 bp UPGMA cluster analysis divided the six cultivars in two main clusters (Yusuf, 2017).

The genetic similarity matrix between lentil accessions was calculated based on Jaccard's similarity coefficient. For ISSR marker, the genetic similarity values of the Jordanian lentil accessions were high between 0.875 and 0.176 within all the accessions, with the most genetically closest accessions were LE 3428 from Irbid and LE 3426 from Balqa (0.875) and the most distant accessions were found between LE 3426 and L 3380 from Balqa (0.176). Comparing the *L. culinaris* accessions alone, the genetic similarity values were between 0.826 and 0.463, the genetically closest accessions were found

between L 3409 and L 3395 from Karak (0.826), while the lowest genetic similarity was found between L 4362 Maan and L 3363 Jerash (0.463) accessions. Comparing the *L. ervoides* accessions together, the genetic similarity value was the highest 0.875 between LE 3428 from Irbid and LE 3426 from Balqa, while the most distant was calculated as 0.240 that was found between LE 3437 from Karak and LE 3412 from Ajlun.

For SCoT marker, the genetic similarity values were between 0.872 and 0.254 within all the Jordanian *Lens* accessions, the most genetically closest accessions were found between LE 3426 from Balqa and LE 3412 from Ajlun (0.872), while the lowest genetic similarity values were found between LE 3426 and L 3377 from Balqa (0.254). Comparing the cultivated accessions alone, the genetic similarity values were between 0.831 and 0.423, as the most genetically closest accessions were found between L 3374 and L 3370 from Madaba (0.831), while the lowest similarity was found between L 3411 from Tafila and L 3379 from Ajlun (0.423). Separated the wild accessions together, the genetic similarity value was the highest 0.872 between LE 3426 from Balqa and LE 3412 from Ajlun, while the most distant similarity was found as 0.297 between accessions LE 3436 from Tafila and LE 3428 from Irbid. The differences in the chromosomal location for ISSR and SCoT primers can influence the diversity assessment, giving different results as shown.

According to the clustering pattern obtained from ISSR analyses, the 35 lentil accessions were grouped into 3 main clusters: cluster I included LE 3412 from Ajlun, LE 3426 from Balqa and LE 3428 from Irbid; cluster II included LE 3429 from Irbid, LE 3435 from Maan, LE 3436 and LE 3438 from Tafila and LE 3437 from Karak, while cluster III included all the cultivated lentil accessions divided into smaller groups. Group I included L 3352, L 3360, L 3388, L 3400, and L 3401 from Irbid province together; group II contained L 3384 from Irbid, L 3404 from Ajloun, L 3374 from Madaba, L 3376, L3377, L3380 from Balqa and L3392 from Karak; group III contained L 3353 and L 3363 from Jerash, L 3365 from Amman, L 3370 and L 3366 from Madaba, and L 3364 from Ajlun; and group IV included L 3439 and L 3379 from Ajlun, L 3390, L 3395 and L 3409 from Karak, L 4362 and L 4538 from Maan, L 3411 from Tafila, L 3362 from Jerash and shown in figure 3.79.

Similarly, the dendrogram produced using SCoT analyses grouped the 35 lentil accessions also into 3 main clusters: cluster I included LE 3412 from Ajlun, LE 3426 from Balqa and LE 3428 from Irbid, cluster II included LE 3429 from Irbid, LE 3435

from Maan, LE 3436 and LE 3438 from Tafila and LE 3437 from Karak, and cluster III included all the cultivated lentil accessions divided into smaller groups. Group I contained L 3352, L 3360 from Irbid, L 3353, L 3362 and L 3363 from Jerash, L 3366, L 3370, and L 3374 from Madaba; group II contained L 3384, L 3388, L3400 and L 3401 from Irbid, L 3404 and L 3379 from Ajlun, L 3395, L 3392, L 3390 and L 3409 from Karak, group III contained L 3376, L 3377 and L 3380 and Balqa and L 3365 from Amman, and group IV contained L 3364 and L 4339 from Ajlun, L 4538 and L 4362 from Maan and L 3411 from Tafila shown in Fig. 3.82.

The ISSR genetic structure of the 35 Jordanian lentil accessions were grouped by the STRUCTURE software clustering model into two clusters K=2 with two different colors red and green, while the red color presented within the 27 cultivated *L. culinaris* accessions, the three unique wild accessions of *L. ervoides* with the codes LE 3412 from Ajlun, LE 3426 from Balqa and LE 3428 from Irbid presented in the green color and the rest five wild accessions LE 3429 from Irbid, LE 3435 from Maan, LE 3437 from Karak, LE 3436 and LE 3438 from Tafila presented in mix with the two clusters together somehow in equal percentage. On the other hand, the SCoT genetic structure grouped the 35 Jordanian lentil accessions into three clusters K=3 divided the wild accessions into two groups instead of one as shown earlier in ISSR analysis, three different colors presented red, green, and blue. In this analysis also all the cultivated *L. culinaris* accessions presented in one cluster with the red color, while the unique three wild accessions (LE 3412 from Ajlun, LE 3426 from Balqa and LE 3428 from Irbid) presented in the green color, and the rest of the five wild accessions presented in different cluster with blue color.

As we adopted 35 lentil accession out of them 8 accessions were related to the wild type *L. ervoides* to study their genetic diversity, genetically we found 3 different populations, where the cultivated accessions were collected in one population in both ISSR and SCoT genetic structure, instead the wild accessions separated in 2 different populations as shown previously in SCoT genetic structure, or were mixed between the two clusters in the case of the five wild types (LE 3429 from Irbid, LE 3435 from Maan, LE 3437 from Karak, LE 3436 and LE 3438 from Tafila) which were genetically mixed between the cultivated and the wild accessions in ISSR genetic structure. Therefore, it can be said that SCoT genetic structure was more informative than ISSR genetic structure in discriminating *L. culinaris* and *L. ervoides* accessions.

Referring to the seed shape of the 35 accessions, the 27 *L. culinaris* cultivated accessions had the same seed features such as the shape and size, the seeds color was ranged between beige, light brown to brown with some dark spots on some of the seeds (Fig. 4.2). While the wild accessions were smaller in size with dark brown color (Fig. 4.3. C and D). Looking more in the details, there were differences in the size, shape, and color between them, as the three wild accessions with the codes LE 3412, LE 3426 and LE 3428 were much smaller, rounder shape and darker brown to black color (shown in Fig. 4.3. in A and B), the same wild accessions which were grouped in separate cluster with the green color.

Figure 4.2. *Seed samples of the cultivated lentil accessions showed their shape and color.*

Figure 4.3. *Seed samples of the wild lentil accessions showed their shape.*

These findings resulted that instead of confirmed only two types of lentil, three different populations were present, 26 accessions recorded as cultivated types under *L. culinaris* and the 27th cultivated accession coded as L 4339 from Ajlun under *L. culinaris* sp. *orientalis* were genetically the same, 3 *L. ervoides* accessions were genetically the same and the rest 5 *L. ervoides* accessions were genetically different than the previous 3 LE accessions. Although the accessions within each population were geographically distant from each other, they were genetically similar revealing that clustering based on genetic similarity was not strongly correlated with the geographic origin, it may indicate that they were from the same ancestor.

Are all the wild accessions being *L. ervoides*? In order to answer this question more research must be done to confirm the phenotypic characteristics for them, more over phenotypic and genotypic tests have to be done to the rest accessions presented in the seed genebank. The known *Lens* species presented in Jordan were *L. culinaris*, *L. culinaris* sp. *orientalis*, and *L. ervoides* as referred in the Jordan Plant Red List Book (Taifour & El-Oqlah, 2015). According to these findings the seed genebank contain three different populations instead of two. More genetic research can be done to screen the rest *Lens* accessions to confirm their genetic origin and structure.

4.3. Genetic Diversity of Almond Accessions

To estimate genetic diversity among 36 almond accessions 13 ISSR and 10 SCoT primers were used. ISSR analyses produced 200 bands of which 188 were polymorphic, with an average of 93 % polymorphism. The number of amplified bands varied between 10 (ISSR 817) and 20 (ISSR 834) with an average of 15 bands per primer. The average PIC value of used ISSR primers was calculated as 0.25. On the other hand, 10 SCoT primers amplified reproducible 193 bands, among these 174 were polymorphic (PPB: 90 %). The number of amplified bands varied between 13 (SCoT 28) and 23 (SCoT 12) with an average of 19.3 bands per primer. The average PIC value of SCoT primers was calculated as 0.25. From these results we can conclude that the number of bands and polymorphism levels of ISSR primers were higher than SCoT primers. The average PIC values of SCoT and ISSR primers were the same (0.25).

Polymorphism percentage values of our study were similar to that of previous studies. For example, Xing et al., (2015) reported that 9 primers of ISSR yielded a total of 66 bands used to study 46 samples of *Amygdalus mira* which were collected from 4 regions in Tibet, China. A mean of 7.3 bands per primer was scored, with 100 %

polymorphic bands. The average PIC value calculated as 0.83. The study indicated that ISSR was an efficient method to reveal genetic diversity for *A. mira* populations. In this study PIC value was indicated as 0.83, since ISSR markers are dominant the maximum PIC value should be 0.50, so PIC value in this study might be miscalculated. Mohamed, Ghareeb, Ibrahim, & Hafez, (2020) showed that 6 ISSR primers produced a total number of 68 bands in 1 genotype of *Juglans regia L.* and 3 genotypes of *Prunus dulcis L.*, among them 51 bands were polymorphic and created 75 % polymorphism. 15 Portuguese almond cultivars were tested using 5 ISSR primers produced 61 reproducible bands, with 12.2 bands per primer which the polymorphic percentage was 96.7% (Martins, Tenreiro, & Oliveira, 2003).

The genetic similarity matrix between almond accessions was calculated based on Jaccard's similarity coefficient. For ISSR markers, the genetic similarity values were high between 0.899 and 0.281. The most genetically closest were found between cultivars Fillis from Greece and Douma 3 from France (0.899), and the great distance was between the wild type *A. arabica* and Ne-Plus Ultra (0.281). Matching the genetic similarity values of the cultivated almond together were between 0.899 and 0.427. The most genetically closest were found between cultivars Fillis and Douma3 (0.899), while the lowest genetic similarity value was found between Oja from Syria and Douma14 from France (0.427). Discussing the genetic similarity values of the wild types were between 0.642 and 0.371 indicated that genetic variation between the studied wild almond accessions was not high, it was moderate level. The most genetically closest accessions were the wild type from Irbid/Malka and the wild type from Al-Ibrahimiye (0.642), and the genetically far distant ones were the wild type *A. arabica* and the wild type from Irbid Naime (0.371).

A lot of sweet almond cultivars showed moderate to high similarity with each other, however, their origin was different. The similarity values between Primorski (Russia) and Ahmah (Syria) was 0.788, between Fillis and Douma3 was 0.899, between SF 121 (Spain) and Ferraduel (France) was 0.729, between Fkusii (Italy) and Texas (USA) was 0.659, between Fkusii (Italy) and A15-5 (Spain) was 0.613, between Ne Plus-Ultra (USA) and A15-5 (Spain) was 0.605, and between Drake (USA) and Kock Tender was 0.656.

In our study, ISSR marker showed a moderate level of genetic similarity existed within the wild almond accessions, as the similarity value between Ajlun/Baun and Ajlun/Mahna types was 0.543, between Ajlun/Baun and Irbid/Naime types was 0.559, between Al-Ibrahimiye and Ajlun/Mahna types was 0.537, between Zarqa and Marj Al-

Furs types was 0.624, between Wadi Shuaib and Marj Al-Furs types was 0.628, between Al-Salt and Wadi Shuaib types was 0.568, between Shobak and Al-Salt types was 0.571, and between Dana District and Shobak types was as 0.581, in addition to the highest value scored previously between Irbid/Malka and Al-Ibrahimiye types was 0.642.

For SCoT marker, the genetic similarity values were between 0.818 and 0.289 within all the 36 almond accessions, the most genetically closest accessions were found between cultivars Fillis from Greece and Douma 3 from France (0.818), and the lowest genetic similarity value was found between A13 and the wild type *A. arabica* (0.289). Comparing the genetic similarity values of the cultivated almond together were between 0.818 and 0.421. The most genetically closest accessions were Fillis and Douma 3 (0.818), while the lowest genetic similarity value was found between A13 and Primorski (0.421). Discussing the genetic similarity values of the wild types were between 0.789 and 0.455, the most genetically closest accessions were the wild type from Dana District and the wild type from Shobak (0.789), while the lowest genetic similarity value was found between the wild type *A. arabica* and the wild type Ajlun/Mahna (0.455), the same value was found also between the wild type *A. arabica* and the wild type from Al-Salt.

SCoT marker showed a moderate to high level of genetic similarity existed within the cultivated almond accessions, as the similarity value between Chellaston (Australia) and Douma 14 (France) was 0.624, between Ferraduel (France) and Fillis (Italy) was 0.621, between Princesse (France) and Chellaston (Australia) was 0.656, Ardshwar86 and Princesse (both from France) was 0.667, between A 4-4 (Spain) and Princesse (France) was 0.778, between A 4-4 (Spain) and Dafadii (Syria) was 0.686, between Fkussi (Italy) and Princesse (France) was 0.667, between Shami fark (Syria) and Ne Plus-Ultra (USA) was 0.667, between Touno (Italy) and Dafadii (Syria) was 0.637, between Dafadii (Syria) and Ferraduel (France) was 0.667, between A4-4 (Spain) and Ahmah (Syria) was 0.63, between Oja (Local) and Ne Plus-Ultra (USA) was 0.648, and between Oja (Local) and Douma 3 (France) was 0.64.

According to the clustering pattern obtained from ISSR analyses, the 36 almond accessions were grouped into three main clusters: cluster I included the wild type *A. arabica*, cluster II included all the wild accessions together divided into three small groups. Group I contained Dana District type with Shobak type (geographically near to each other, south of Jordan), group II contained Al-Salt, Zarqa, Wadi Shuaib and Marj Al-Furs types (geographically presented in the middle part of Jordan), and group III

contained Irbid/Malka, Al-Ibrahimiye, Ajlun/Mahna, Irbid/Naime, and Ajlun/Baun types (geographically located in the north part of Jordan). Cluster III included all the 24 cultivated accessions together divided into three small groups; group I included Oja (Local), Dafadii (Syria), Ardshwar 86 (France), Ai (France), and Douma14 (France). Group II included A13 (Spian), Tuono (Italy), Ne Plus-Ultra (USA), Douma 25 (Syria), A4-4 (Spian), A15-5 (Spain), Princesse (France), Fkusii (Italy), Texas (USA), and Chellaston (Australia). Group III contained Shami Fark (Syria), Fillis (Greece), Douma 3 (France), Drake (USA), Kock Tender (France), SF 121 (Spian), Ferraduel (France), Primorski (Russia), Ahmah (Syria).

On the other hand, the dendrogram produced using SCoT analyses grouped the almond accessions into three main clusters: cluster I was included only the wild type *A. arabica*, cluster II included all the rest wild accessions together divided also into three small groups. Group I contained Zarqa and Marj Al-Furs types together (geographically located in same province limits), group II included Dana District, Shobak, Al-Salt, Wadi Shuaib types (located in the same biogeographical region), and group III contained the northern types together Irbid/Malka, Ajlun/Mahna, Al-Ibrahimiye, Irbid/Naime, and Ajlun/Baun. Cluster III included all the 24 cultivated accessions together separated into four small groups, group I included Primorski (Russia), Drake (USA), Texas (USA), Douma 25 (Syria), and SF 121 (Spian). Group II contained Ai (France), Ardshwar 86 (France), A4-4 (Spian), A15-5 (Spain), Princesse (France), Fkusii (Italy), Dafadii (Syria), and Ferraduel (France). Group III included Tuono (Italy), A13 (Spian), Fillis (Greece), Douma 3 (France), Chellaston (Australia), Douma14 (France). Group IV contained Kock Tender (France), Oja (Local), Ne Plus-Ultra (USA), Shami Fark (Syria), and Ahmah (Syria).

In our study, wild types of almond accessions were grouped separately from cultivated sweet almond accessions with both ISSR and SCoT analyses. Similarly, Shiran et al. (2007) mentioned that *P. orientalis* and *P. scoparia* (two almond wild species) were clustered out of the cultivated *P. dulcis*. Some of the accessions were clustered together according to the origin and some according to the proximity, although the wild types were clustered in the same cluster but grouped according to regions, as the northern types grouped together (Mediterranean region), while the southern types grouped in different section (Irano-turanian region) in ISSR dendrogram tree. Al-Hmoud, Hamasha, & and Al-Kayed, (2003) mentioned that *A. communis* presented in the Mediterranean region,

while *A. korschinskyi* presented in the Irano-turanian region south of Jordan. According to that *A. korschinskyi* located in Dana District and Al-Shobak as located in the south and grouped together separately from the rest genotypes. Finding that ISSR dendrogram tree was more informative than SCoT tree. In addition, *A. arabica* which is a wild almond shrub grown in Al-Azraq desert or in the Saharo-Arabian biogeographical region, totally different from the other types of wild almond trees and clustered alone in ISSR and SCoT dendrogram trees indicating reliability of our results.

Although their origin was different, a lot of sweet almond cultivars used in our study showed moderate to high similarity and they were clustered together, but this clustering pattern was not relevant to their origin. The Syrian types presented in mix with all the types of Greece, France, Spain, Italy, Russia, USA and Australia. The Australian type (Chellaston) was grouped far away from the Russian type (Primorski) in ISSR and SCoT analyses. The French, Spanish and American cultivars were grouped together with the other almond cultivars as shown in ISSR and SCoT analyses. Types Douma 3 and Douma 14 said to be originated from France (in the origin table given from Al-Mushaqar Station), while were with unknown origin in another reference (Al-Hmoud, Hamasha, & and Al-Kayed, 2003), the name "Douma" is related to a place in Syria while also Douma 25 is a Syrian type, may be they were breeding types from the same rootstock. However, Oja which became a local variety in Jordan after importing it from Syria decades ago, shows a close relationship to the Jordanian wild types in the ISSR dendrogram tree of genetic similarity, as it was proved in previous study done by La (2009) using RAPD analysis, which said that Oja cultivar was the most distant compared to the rest sweet cultivars.

Almond originated in Central Asia and over several centuries it was spread to the west towards the Mediterranean Basin. Nowadays it is present mainly grown in the Mediterranean Basin, the Middle East, south-western Asia, USA, and Australia (Martí et al., 2015). Almond cultivars of the Mediterranean origin have been widely spread to other sites reached Australia and USA. However, almond breeders have done some local selections and breeding work. Some American almond cultivars such as I.X.L., Texas, Ne Plus Ultra, and Nonpareil were originated from a single seedling orchard planted by A.T. Hatch in California1879, and all of them derived from the seedling material of Languedoc region in France (Xu, Ma, Xie, Liu, & Cao, 2004). The French and American cultivars genetically related to each other according to provided information.

Fernández i Martí, Font i Forcada, Kamali, Rubio-Cabetas, & Wirthensohn, (2015) mentioned in their study that the domestication of almond in Central Asia was probably the result of hybridizations among several species, which was happened in most species, represented the first loss of genetic diversity that later increased by the spread of almond towards different growing regions, as each local population could have been obtained from a reduced number of introductions, such as the following cases: California and Australia. Almond was introduced in the 1800's to California (Mediterranean-like climate) by the early Spanish missionaries, and the 'Jordan' type was the most popular type imported from Spain, which was a hard-shell one, as most of the Spanish cultivars. In addition to 'Jordan' type in California many cultivars such as 'Fines des Dames', 'Nonpareil', 'I.X.L.' and 'Ne Plus Ultra' presented. However, intensive breeding between varieties has narrowed the genetic diversity, since most cultivars came from California (same place) and originated from a small gene pool. Around year 1837 almond trees of 'Jordan' type were imported by horticulturists and planted throughout Adelaide, Southern Australia. Australian cultivars have been poorly studied to date, and the varieties are supposed to be closely related and difficult to distinguish morphologically.

All the cultivars coming from USA and Australia were spread from the Mediterranean Basin as Spain, Greece, France, Italy, Syria and Jordan were a part of the Mediterranean region, so that the cultivars were located and mixed together in the same cluster, in addition to that almonds are highly heterozygous and highly polymorphic because of its strong self-incompatibility system and predominancy to open pollination (Mir and Patel, 2018), that's why this study showed a high level of genetic similarity values existed in the cultivated almond accessions, while the genetic similarity values were low comparing the cultivated types with the wild types as they were grown in different areas. According to study made by La (2009) using RAPD marker, proved the existence of genetic variation among 16 sweet-cultivated types and wild Jordanian types and they were separated in different groups.

The ISSR genetic structure of 36 almond accessions were grouped by the STRUCTURE software clustering model into three clusters K=3 with three different colors blue, green, and red. All the 24 cultivated types were clustered together in blue color. The wild types were clustered in the green group except one wild type *A. arabica* clustered alone in the red cluster. Same results were observed by La (2009) and Shiran, et al., (2007) where the wild landraces were placed in different cluster away from the

cultivated types. Some of the cultivated and wild types were genetically related to *A. arabica* as the red cluster blended in the rest clusters. Douma 14 was almost 55 %, Ardshwar was 35 %, Dafadii was 55 %, Touno was 30 %, Ai was 40 %, Wild Irbid/Naime was 10 %, Wild Irbid/Malka was 10 % and wild Dana District was 20 % related to the wild type *A. arabica*. The wild types Ajlun/Mahna and Irbid/Naime had 10 % and 20 % respectively of their parts with the blue cluster related to the cultivated types.

From that a conclusion can be said that these cultivars could have a root stock of the wild ancestor *A. arabica* or their parents (ancestors or root stock) were grown in near area were *A. arabica* presented. The genetic combination between almond species promoted by several reasons; self-incompatibility increases the option of cross pollination and pollen transferers as wind, bees, or insects even animals can help. Pollens of the *A. arabica* were transferred to other almond species, after a while these species were propagated or some of their fruits were taken to another place, replant them then grown and so one. This how the genes can spread across and be exchanged. La (2009) mentioned that the presence of wild crop relatives causes the evolution of novel genetic variety and the ongoing gene flow between and among plant species. As an example, Touno from an Italian origin was thought to be an offspring from ancient hybrid between *P. dulcis* and *P. webbii* (Martins, Tenreiro, & Oliveira, 2003), may be one of its ancestors contained genes from *A. arabica*, which transferred to the cultivated type Touno. Results were observed by La (2009) and Shiran et al. (2007) that the wild landraces believed to be the origin of almond cultivars.

On the other hand, the SCoT genetic structure grouped the 36 almond accessions into only two clusters K=2 with two different colors, the red cluster for the 24 cultivated types and the green cluster for all the 12 wild types including *A. arabica*, without any actual combination could be mentioned. From the previous explanation we can conclude that the genetic structure in addition to the dendrogram tree of ISSR marker was more informative than SCoT marker in genetic analysis of almond.

5. CONCLUSION

This study is the first to consider genetically on the adopted accessions from NARC and will give a rich information for future studies as chickpea and lentil accessions were imported from their seed bank under the International Treaty on Plant Genetic Resources for Food and Agriculture (SMTA).

The 18 chickpea accessions were from the kabuli type and genetically similar even that they were geographically distant landraces, except the accessions from Amman, Mafraq and Balqa provinces that were genetically different according to SCoT analyses. As most of the accessions were the same with some of primers but they were not identical. Therefore, we cannot say that there is a duplication in the seed genebank for chickpea seeds.

The 35 lentil accessions genetically presented in 3 diverse populations instead of two according to our findings, as the 27 cultivated *L. culinaris* located in one cluster, while the wild *L. ervoides* divided into two clusters according to SCoT analyses, regardless to their geographic origin. In this study we have only used 35 accessions out of 97 presented in the seed genebank. Among the studied accessions there was no duplication, however studies should be undertaken with the rest of accessions with the same markers and PCR conditions.

36 Almond accessions were grouped into 3 clusters with ISSR marker while in SCoT marker grouped the cultivated types in one and the wild types in another cluster, regardless of their geographical origin. According to our findings the clustering based on genetic similarity was not strongly correlated with the geographic origin and it can be said that each cluster/ population came from the same ancestor.

Molecular markers are stable, quick, and reliable techniques can be applied in laboratory conditions. ISSR and SCoT molecular markers both are dominant, high reproducible, little of DNA amount needed and easy to work with, made them a useful source for studying plant genetic population and diversity. From our study ISSR marker was more informative than SCoT marker in examining almond accessions, while SCoT marker was more preferable than ISSR marker for genetic diversity studies in chickpea and lentils.

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CURRICULUM VITAE

Name: Ghina Al-Hmoud

Education

- **2018-2022**

PhD in Botany, University of Anatolia / Eskisehir technical University, Eskisehir, Turkey
Dissertation title "*Determination of Genetic Variations of Chickpea, Lentil and Almond Genotypes Stored in the Jordanian Gene Bank Using PCR-Based Molecular Markers*".
Holder of the YTP scholarship.

- **2010-2013**

Master's in Plant Protection, University of Jordan, Amman, Jordan
Thesis Title "*Effect of Different Commercial Mycorrhizal Products on Plant Growth and Fusarium Root Rot of Different Vegetable Crops*".

- **2005-2009**

Bachelor's in plant Protection, University of Jordan, Amman, Jordan
Graduated head of the 2009 class and was awarded a full scholarship to continue my master's education.

Experience

- **2015-2017**

Teaching Assistant, Department of Biology, University of Jordan, Amman, Jordan
Taught the General Biology, Botany, and Plant Physiology laboratories.

- **2009-2015**

Research and Teaching Assistant, Department of Plant Protection, University of Jordan, Amman, Jordan.
•Taught the General Plant Pathology, Fungal Plant Diseases, Mycology, Economic Entomology, and Breeding of Honeybees laboratories.
• Assisted faculty in their research, laboratory experiments, and documentation and publications.

•Sample collection, sorting, identifying, and testing of plants and plants pathogens.

Publications

•Al-Hmoud, G. and Al-Momany, A., (2015). *Effect of Four Mycorrhizal Products on Fusarium Root Rot of Different Vegetable Crops*. Journal of Plant Pathology & Microbiology, 6:2.

• Al-Hmoud, G., and Al-Momany, A., (2017). *Effect of Four Mycorrhizal Products on Squash Plant Growth and Its Effect on Physiological Plant Elements*. Advances in Crop Science and Technology Journal, 5:1.

• Al-Hmoud, G., and Al-Momany, A., (2020). *Effect of Four Mycorrhizal Products on Onion Growth*. Fresenius Environmental Bulletin, 29:4.

Projects

- **2020-2022**

Working with my PhD project

Identifying the genetic consumption and diversity of some accessions of chickpea, lentil, and almond with local and wild Jordanian varieties using ISSR and SCoT molecular markers.

- **2013 – 2014**

"**Wild Mushroom in Jordan**" Book Translation

Assisted Prof. Dr. Ahmad Al-Momany in the translation of his book "Wild Mushroom in Jordan" from English to Arabic.

- **2010 – 2012**

Expanding the Mycorrhizae Project

Built upon on my master's thesis research work to further verify the scientific results of effect the Mycorrhizae on the Fusarium root rot and expanded the working scope of the research on different crops for two years.

Technical Skills

Experienced in filed and laboratory-based plant and plant related pathogen sampling, sorting, extraction, purification, testing and statistical analysis, including:

• CTAB-based plant DNA extraction.

• Gene extraction using PCR-based molecular markers.

• Gel electrophoresis-based DNA analysis, Nanodrop Spectrophotometer

• SPSS analysis.

• Total Lab CLIQS 1D, DendroUPGMA program, MEGA 11 software program.

• Light compound microscopes and stereoscopic microscopes.

• Preparing temporary plant tissue slides.

Professional Activities

Professional Workshops:

• Participated in the International Summer School Program "Promoting Conservation Agriculture in the Arab Region" At Agriculture Research and Education Center at the American University of Beirut, Lebanon. Sponsored by the German Academic Exchange Service (DAAD), 2011.

Training Courses:

• "Food as Medicine", Monash University, UK. Three weeks long, virtual, 2017.

• "The Science of Nutrition", The Open University, UK. Four weeks long, virtual, 2017.

• "Invasive Fruit Tree Pests Surveillance in the Mediterranean Region" managed by MAIB (Mediterranean Agronomic Institute of Bari) 2013.

• "Post-harvest", USAID, SABEQ Program, Amman, Jordan 2008.

• "Communication Skills", USAID, SABEQ Program, Amman, Jordan 2008.

• "Development of Educational Skills", Human resources Development Center, University of Jordan 2010.

Professional Memberships

•Jordan Agricultural Engineers Association (JAEA).

•The Arab society for plant protection (ASPP).

Language

•Arabic: mother tongue.

•English: very good in reading, writing and communication skills.

•Turkish: C1 certified.