ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Md Rashedul ISLAM

CONSTRUCTION OF HIGH-DENSITY GENETIC LINKAGE MAP AND QTL ANALYSIS USING AN INTERSPECIFIC F_1 POPULATION IN PISTACHIO

DEPARTMENT OF BIOTECHNOLOGY

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We certify that the thesis titled above was reviewed and approved for the award of the degree of Doctor of Philosophy (PhD) by the board of Juries on 06/03/2020.

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ABSTRACT

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Pistachio (*Pistacia vera* L.) is one of the commercially most important cultivated fruit tree species around the world including Turkey due to its edible nuts having beneficial properties to human health as well as commercial importance in the industry. Cultivar breeding programs in pistachio take a long time, huge labor, high cost, and large land areas due to the alternate bearing and long juvenile period of pistachio. To mitigate such constraints in the breeding programs, the molecular approach is necessary to apply. Therefore, this study aimed (i) to construct the first SNP-based high-density genetic linkage maps using an interspecific F_1 population derived from a cross between *P. vera* cv. 'Ohadi' and *P. atlantica* cv. 'Pa-18' ('Ohadi x Pa-18'), (ii) to perform QTL analysis in leaf, shoot and inflorescence traits in pistachio. To obtain the SNP markers, DArT-Seq^{TM} genotyping-by-sequencing method was used. A total of 1,407 SNPs was mapped in the 'Ohadi' female, whereas the 'Pa-18' male map consisted of a total of 2,518 SNPs markers. The total length of the 'Ohadi' map was 1,122.2 cM with an average length of 74.8 cM, while the 'Pa-18' map was 1,307.2 cM in length with an average length of 87.1 cM. The marker density was 1.2 and 1.9 markers per cM in 'Ohadi' and 'Pa-18' linkage maps, respectively. 'Ohadi' map had an average of 93.8 markers per linkage group, while the 'Pa-18' map had an average of 167.9 markers per linkage group. A total of four significant QTLs that are related to terminal leaflet base, leaf width, tree vigor, and inflorescence rachis length were detected in three linkage groups in the 'Ohadi' genetic linkage map. The data generated in this study will be very useful and framework for further QTL analysis, molecular breeding, and genetic studies in the future for pistachio.

Key words: Pistachio, *Pistacia vera*, Genetic mapping, SNP, QTL

ÖZ

DOKTORA TEZİ

ANTEPFISTIĞI TÜRLERARASI F_1 POPULASYONUNDA YÜKSEK YOĞUNLUKTA GENETİK HARİTANIN ELDESİ VE QTL ANALİZLERİ

Md Rashedul ISLAM

ÇUKUROVA ÜNİVERSİTESİ
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Antepfıstığı (*Pistacia vera* L.), insan sağlığına faydalı ve ticari olarak sanayide önemli yeri olan, yenilebilir kabuklu meyvelerden biridir. Türkiye'de dahil olmak üzere dünyanın en önemli ağaç türlerinden biridir. Antepfıstığında ıslah programlarının uzun zaman alması, daha fazla emek istemesi, yüksek maliyet ve büyük arazi alanlarını işgal etmesi antepfıstığnın periyodisite ve uzun gençlik kısırlığı dönemi ile ilgili sorunlardan kaynaklanmaktadır. Islah programlarında bu tür kısıtlamaların üstesinden gelmek için moleküler yaklaşımlar gerekmektedir. Bu nedenle, bu çalışmanın amacı (i) *P. vera* cv. 'Ohadi' ve *P. atlantica* cv. 'Pa-18' ('Ohadi x Pa-18') arasında yapılan melezlemeden oluşturulmuş F_1 populasyonu kullanılarak ilk SNP tabanlı yüksek yoğunluklu genetik bağlantı haritalarının oluşturulması ve (ii) antepfıstığında yaprak, sürgün ve çiçek salkımı özellikleri üzerine QTL analizlerinin yapılması amaçlanmıştır. Dişi 'Ohadi' çeşidinde toplam 1.407 SNP haritalanırken, erkek 'Pa-18' haritası toplam 2.518 SNP'ten oluşmuştur. 'Ohadi' haritasının toplam uzunluğu 1.122,2 cM ve ortalama uzunluğu 74,8 cM iken, 'Pa-18' haritasının toplam uzunluğu 1.307,2 cM ve ortalama uzunluğu 87,1 cM'dir. 'Ohadi' ve 'Pa-18' bağlantı haritalarında markör yoğunluğu sırasıyla cM başına 1,2 ve 1,9 markör bulunmuştur. 'Ohadi' haritasında bağlantı grubu başına ortalama 93,8 markör haritalanırken, 'Pa-18' haritasında ise bağlantı grubu başına ortalama 167,9 markör haritalanmıştır. 'Ohadi' haritasındaki üç bağlantı grubunda uç yaprakçık taban şekli, yaprak genişliği, ağaç kuvveti ve çiçek salkımı sapı uzunluğu ile ilgili toplam dört önemli QTL bulunmuştur. Bu araştırmadan elde edilen veriler antepfıstığı için gelecekte QTL analizi, moleküler ıslah ve genetik çalışmalar için çok yararlı olacaktır.

Anahtar Kelimeler: Antepfıstığı, *Pistacia vera*, Genetik harıtalama, SNP, QTL

EXTENDED ABSTRACT

Pistachio is one of the commercially most important nuts that is widely cultivated in Turkey. Every year, more farmers are showing interest to cultivate it. Thus, pistachio production in Turkey has been growing for several decades. The scenario is also true for other major producing countries like the USA and Iran. Therefore, in the last 15-20 years, the production of pistachio nuts in the world has been significantly increased. Especially, after knowing the important benefits of pistachio nuts to human health and its uses in the industry, more attention has been given to it.

Pistachio is known as the "green gold tree" for its commercial and nutritional importance. Pistachio has a high nutritional value including high protein, vitamin, mineral, and unsaturated fat content that are beneficial to human health. Pistachio kernels have both monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The unsaturated fatty acids are a nutritional provision for humans due to their health-related advantages. Essential fatty acids are decisive for the human diet for the preservation of growth and reproduction. MUFAs, such as oleic acid, has properties in decreasing plasma triacylglycerol and cholesterol concentrations in human blood. Likewise, PUFAs, such as linoleic and linolenic acids play roles in preventing cancer, atherosclerosis, heart disease, and diabetes. Pistachio shows nutritional advantages in significantly decreasing blood cholesterol. A 20% of the consumption of pistachio nuts of daily-basis caloric intake edges to remarkable improvement in HDL (high-density lipoprotein) and inhibits LDL cholesterol oxidation in human blood. Pistachio has beneficial properties for prevention as well as the treatment of coronary artery diseases. This is due to having the high levels of both MUFAs and PUFAs, and probably for the tocopherol content in pistachio.

The producers select cultivars with high productivity and high quality. The yield of pistachio nuts is directly proportional to the nut yield on the branches of the tree. While selecting the cultivars, nut size, filled interior, hard shell, and interior color are the most important nut characteristics to be considered. Besides, late-flowering is also one of the most important criteria in order not to be damaged by the late spring frost.

With the advancements of molecular marker technique that emerged in the late 1990s, numerous studies were carried out to improve the properties of pistachio. These studies facilitated the collection of information on agricultural properties such as molecular breeding, genetic diversity, yield, and some sort of pistachio biochemistry. The scientific research on the plant species and the development of molecular methods of application reveal a new perspective both in selection processes and in evolutionary studies.

Although genetic linkage mapping studies have been made intensively by using many different types of molecular marker techniques, the study on pistachio is not sufficient to reveal certain mechanisms including alternate bearing and long juvenile period. Moreover, the study on pistachio using SNP marker system is extremely limited. While observing the studies of linkage mapping and QTL analysis in pistachio, the QTL studies are very limited in the literature. For these reasons, it is necessary for extensive genetic mapping and QTL studies in pistachio.

The construction of genetic linkage maps is considered as the beginning point of several downstream studies like QTL, MAS, development of molecular markers, gaining a genome sequence at a chromosomal level. Several molecular marker techniques including AFLP, RAPD, RFLP, SSR, ISSR, and SNP were employed in pistachio to understand the underlying molecular mechanisms. However, the construction of genetic linkage maps in pistachio in a few previous studies was very limited that did not eventually reveal the complete scenario of pistachio chromosomes and its molecular structure. In this current study, we tried to

overcome the pitfalls of previously published studies and to get a densely constructed genetic linkage map by utilizing the highest numbers of SNP markers. We aimed to collect the genetic information lying inside the 15 haploid pairs of chromosomes of pistachio to switch the classic breeding into molecular breeding. Eventually, we constructed the first high-density genetic linkage maps in pistachio from an interspecific F_1 population of 'Ohadi x Pa-18' cultivars using SNP markers in this study.

In many fruit species, such as pistachio, the breeding programs take a long time. The most important reason for this is the fact that pistachio has a long juvenile period and alternate bearing tendency. In a classical breeding program, F_1 breeding plots are prepared and progenies are expected to produce nuts. Producers take care of the plot for irrigation, chemical spraying, fertilization, weed control, etc. are carried out for the healthy growth and development of thousands of progenies for many years. In recent years, however, biotechnology has provided opportunities to develop DNA markers associated with economically important characters. This situation leads to the transition from classical to molecular breeding with less cost and in a shorter time and has given a possibility to develop high-quality varieties with high yield.

In the scope of this study, high-density genetic linkage maps with SNP markers have been constructed using an interspecific F_1 population of 'Ohadi x Pa-18'. To obtain the SNP markers, DArT-Seq™ genotyping-by-sequencing method was used. A total of 1,407 SNPs was used to construct the 'Ohadi' female map from 2,112 SNPs, whereas the 'Pa-18' male map composed of a total of 2,518 SNPs from 3,062 SNPs obtained from DArT-Seq™. The total length of the 'Ohadi' map was 1,122.2 cM with an average length of 74.8 cM, while the 'Pa-18' map was 1,307.2 cM in length with an average length of 87.1 cM. The marker density was 1.2 and 1.9 markers per cM in 'Ohadi' and 'Pa-18' linkage maps, respectively. 'Ohadi' map had

an average of 93.8 markers per linkage group, while the ‘Pa-18’ map had an average of 167.9 markers per linkage group.

Within the scope of this current study, a total of 18 phenotypic characteristics related to leaf, shoot, and flowering traits were observed in the F_1 population. Either measurement or observations on leaf length (LL), leaf width (LW), number of leaflets (NL), terminal leaflet length (TLL), terminal leaflet width (TLW), terminal leaflet shape (TLS), terminal leaflet size (TLSZ), terminal leaflet apex (TLA), terminal leaflet base (TLB), tree vigor (TV), one-year-old shoot color (OYOSC), date of first bud burst (DFBB), inflorescence abundance (IA), first bloom date (FBD), peak bloom date (PBD), last bloom date (LBD), date of vegetative bud break (DVBB), and inflorescence rachis length (IRL) were performed in the F_1 progenies in 2018 and 2019 growing seasons.

A total of four significant QTLs that are related to the shape of the terminal leaflet base, leaf width, tree vigor, and inflorescence rachis length were identified on three linkage groups (LG1, LG12, and LG13) in the ‘Ohadi’ map. However, no significant QTLs were identified in the ‘Pa-18’ map. The QTLs that were detected at least in two of the growing seasons were considered significant.

Therefore, the first SNP-based high-density genetic linkage maps of pistachio (‘Ohadi x Pa-18’) were constructed in this study. The linkage maps along with the QTL results obtained in this study will be very useful and framework for further QTL analysis, molecular breeding studies, and genetic studies of pistachio in the near future.

GENİŞLETİLMİŞ ÖZET

Antepfıstığı, Türkiye'de yaygın olarak yetiştirilen en önemli sert kabuklu meyve türlerinden biridir. Her yıl, daha fazla çiftçi antepfıstığı yetiştiriciliğne ilgi göstermektedir. Böylece, Türkiye'nin son yıllarda antepfıstığı üretim kapasitesi artmıştır. Bu durum ABD ve İran gibi diğer büyük üretici ülkeler için de geçerlidir. Bu nedenle, son 15-20 yılda, dünyadaki antepfıstığı üretimi önemli ölçüde artmıştır. Özellikle, antepfıstığının insan sağlığına önemli faydaları ve sanayideki kullanımları ortaya çıktıktan sonra, bu meyve türüne daha fazla önem verilmiştir.

Antepfıstığı, ticari ve besinsel önemi nedeniyle "yeşil altın" olarak bilinir. Antepfıstığı, insan sağlığı için faydalı olan yüksek protein, vitamin, mineral ve doymamış yağ içeriği dahil olmak üzere yüksek bir besin değerine sahiptir. Antepfıstığı hem tekli doymamış yağ asitleri (MUFA'lar) hem de çoklu doymamış yağ asitlerini (PUFA'lar) içerir. Doymamış yağ asitleri, sağlıkla ilgili avantajlarından dolayı insan için önemli bir besin kaynağıdır. Esansiyel yağ asitleri, büyüme ve üremenin korunmasında insan beslenmesi için belirleyicidir. Oleik asit gibi MUFA'lar, insan kanında plazma triasilgliserol ve kolesterol konsantrasyonlarını azaltma özelliklerine sahiptir. Benzer şekilde, linoleik ve linolenik asitler gibi PUFA'lar kanser, ateroskleroz, kalp hastalığı ve diyabetin önlenmesinde rol oynar. Antepfıstığı, kolesterolü önemli ölçüde azaltır. Günlük bazda kalori alımında fıstık ve fındık tüketiminin %20'si, HDL'de (yüksek yoğunluklu lipoprotein) kayda değer iyileşme sağlar ve insan kanında LDL kolesterol oksidasyonunu engeller. Antepfıstığı, koroner arter hastalıklarının yanı sıra korunma için de yararlı özelliklere sahiptir. Bunun nedeni, hem MUFA'ların hem de PUFA'ların yüksek seviyelerine ve muhtemelen fıstıktaki tokoferol içeriğinden kaynaklanmaktadır.

Üreticiler yüksek verimlilik ve yüksek kalitede çeşit seçemektedirler. Antepfıstığı verimi, ağacın dalları üzerindeki meyve verimi ile doğru orantılıdır. Antepfıstığı çeşitleri seçilirken, meyve büyüklüğü, iç doluluk, sert kabuklu ve iç rengi dikkate alınması gereken en önemli meyve özellikleridir. Ayrıca geç yapraklanma, ilkbahar geç donlarından zarar görmemesi için önemli bir seçim kriteridir.

1990'ların sonunda moleküler marörlerin gelişmesiyle, antepfıstığının özelliklerini geliştirmek için çok sayıda çalışma yapılmıştır. Bu çalışmalar moleküler ıslah, genetik çeşitlilik, verim ve biyokimyasal çalışmalar gibi tarımsal özellikler hakkında bilgi elde edilmesini kolaylaştırmıştır. Bitki türleri üzerine yapılan bilimsel araştırmalar ve moleküler uygulama yöntemlerinin geliştirilmesi, hem seçim süreçlerinde hem de evrimsel çalışmalara yeni bir bakış açısı ortaya koymaktadır.

Genetik haritalama çalışmaları birçok farklı moleküler markör tekniği kullanılarak yoğun bir şekilde yapılmış olmasına rağmen, antepfıstığı ile ilgili yapılan çalışmalar, periyodisite ve uzun gençlik kısırlığı dönemi dahil olmak üzere bazı mekanizmaları ortaya çıkarmak için yeterli değildir. Ayrıca, SNP markör sistemi kullanılarak antepfıstığı üzerine yapılan çalışmalar son derece sınırlıdır. Antepfıstığı genetik haritalama çalışamaları ve QTL çalışmaları literatürde çok sınırlı sayıda bulunmaktadır. Bu nedenlerden dolayı, antepfıstığında kapsamlı genetik haritalama ve QTL çalışmalarının yapılması gereklidir.

Genetik bağlantı haritalarının oluşturulması, QTL, MAS, moleküler markörlerin geliştirilmesi, kromozomal düzeyde bir genom dizisi kazanılması gibi çeşitli çalışmaların başlangıç noktası olarak kabul edilir. Altta yatan moleküler mekanizmaları anlamak için antepfıstığında AFLP, RAPD, RFLP, SSR, ISSR ve SNP gibi bir dizi moleküler markör teknikleri kullanılmıştır. Bununla birlikte, önceki bazı çalışmalarda antepfıstığında genetik bağlantı haritalarının oluşturulması çok sınırlıdır. Sonuçta antepfıstığı kromozomlarının ve moleküler yapısının tüm yapısı ortaya çıkarılamamıştır. Bu çalışmada, daha önce yayınlanmış çalışmalarda

yapılmamış olan ve en fazla sayıda SNP markörü kullanarak yoğun bir şekilde oluşturulmuş genetik bağlantı haritası oluşturulması hedeflenmiştir. Klasik ıslahından moleküler ıslahına dönüştürmek için 15 haploid antepfıstığı kromozom çiftinin altında yatan genetik bilginin belirlemesi amaçlanmıştır. Sonuçta, bu çalışmada SNP markörleri kullanarak 'Ohadi x Pa-18' çeşitlerinin F_1 popülasyonundan antepfıstığındaki ilk yüksek yoğunluklu genetik bağlantı haritalarını oluşturulmuştur.

Antepfıstığı gibi birçok meyve türünde ıslah programları uzun zaman alır. Bunun en önemli nedeni, antepfıstığın uzun bir kısırlılık dönemi ve periyodisite eğilimine sahip olmasıdır. Klasik bir ıslah programında melezlemeden sonra, F_1 ıslah parselleri oluşturulur ve melez bireylerin meyve vermesi beklenir. Uzun yıllar binlerce melez bitkinin sağlıklı büyümesi ve gelişmesi için sulama, ilaçlama, gübreleme, yabancı ot kontrolü vb. kültürel bakım işlemlerinin yapılması gereklidir. Bununla birlikte, son yıllarda biyoteknolojik çalışmalar, önemli karakterlerle ilişkili DNA markörleri geliştirme olanağı sağlamıştır. Bu durum daha düşük maliyetle ve daha kısa sürede klasik ıslahtan moleküler ıslaha geçişe yol açmakta ve çeşit geliştirme imkânı vermektedir.

Bu çalışma kapsamında, türler arası bir F_1 popülasyonu olan 'Ohadi x Pa-18' kullanılarak SNP markörleri ile oldukça yoğun bir genetik bağlantı haritası oluşturulmuştur. SNP markörlerini elde etmek için, sekanslama yoluyla DArT-Seq™ genotipleme yöntemi kullanılmıştır. DArT-Seq™'den elde edilen 2.112 SNP markörden dişi 'Ohadi' haritasını oluşturmak için toplam 1.407 SNP haritalanırken, 'Pa-18' haritası başlangıçta elde edilen 3.062 SNP markörden 2.518 adedini içermiştir. 'Ohadi' genetik haritasının toplam uzunluğu 1.122,2 cM ve ortalama uzunluğu 74,8 cM iken, 'Pa-18' genetik haritasının toplam uzunluğu 1.307,2 cM ve ortalama uzunluğu 87,1 cM olmuştur. 'Ohadi' ve 'Pa-18' bağlantı haritalarında markör yoğunluğu sırasıyla cM başına 1,2 ve 1,9 markör olarak hesaplanmıştır.

‘Ohadi’ haritasında bağlantı grubu başına ortalama 93,8 markör haritalanırken, ‘Pa-18’ haritası bağlantı grubu başına ortalama 167,9 markör içermiştir.

Bu çalışma kapsamında, F_1 popülasyonunda yaprak, sürgün ve çiçeklenme özellikleri ile ilgili toplam 18 fenotipik özellik gözlemlenmiştir. Yaprak uzunluğu (LL), yaprak genişliği (LW), broşür sayısı (NL), terminal broşür uzunluğu (TLL), terminal broşür genişliği (TLW), terminal broşür şekli (TLS), terminal broşür boyutu (TLSZ) terminal broşür apeksi (TLA), terminal broşür tabanı (TLB), ağaç canlılığı (TV), bir yıllık çekim rengi (OYOSC), ilk tomurcuk patlaması tarihi (DFBB), çiçeklenme bolluğu (IA), ilk çiçeklenme tarih (FBD), tepe tomurcuğu çiçeklenme tarihi (PBD), son çiçeklenme tarihi (LBD), vejetatif tomurcuk kırılma tarihi (DVBB) ve çiçek salkımı sapı uzunluğu (IRL), 2018 ve 2019 büyüme mevsimlerinde gözlemlenmiştir.

‘Ohadi’ haritasında üç bağlantı grubunda (LG1, LG12 ve LG13) uç yaprakçık tabanın şekli, yaprak genişliği, ağacın büyüme gücü ve çiçek salkımı sapı uzunluğu ile ilgili toplam dört önemli QTL belirlenmiştir. Bununla birlikte, ‘Pa-18’ haritasında önemli QTL tespit edilmemiştir. En az iki büyüme sezonunun tespit edilen QTL’ler önemli olarak kabul edilmiştir.

Sonuç olarak, bu çalışmada antepfıstığında ilk SNP temelli yüksek yoğunlukta genetik bağlantı haritaları oluşturulmuştur. Bu araştırmada elde edilen QTL sonuçları ile birlikte bağlantı haritaları, gelecekte daha fazla QTL analizleri, moleküler ıslah ve antepfıstığında genetik çalışmaları için çok yararlı bir veritabanı oluşturacaktır.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C : degree celsius
A : Adenine
ABI : Applied Biosystems
AFLP : amplified fragment length polymorphism
AMOVA : analysis of molecular variance
BC : back cross
bp : base pair
C : Cytosine
cDNA : complementary DNA
cm : centimeter
cM : centimorgan
cpDNA : chloroplast DNA
CTAB : cetyltrimethylammonium bromide
cv. : cultivar
DArT : Diversity Arrays Technology
dATP : deoxyadenosine triphosphate
dCTP : deoxycytidine triphosphate
dGTP : deoxyguanosine triphosphate
DHLs : double haploid lines
DNA : deoxyribonucleic acid
dNTP : deoxy-nucleoside triphosphate
dTTP : deoxythymidine triphosphate
EDTA : ethylenediaminetetraacetic acid
EST-SSR : expressed sequence tag-derived SSR
FAO : Food and Agricultural Organization of the United Nations
FCA : factorial correspondence analysis

g : gram
G : Guanine
Gb : Giga base
GBS : genotyping-by-sequencing
ha : hectare
HCl : hydrochloric acid
HDL : high-density lipoprotein
H_e : expected heterozygosity
H_o : observed heterozygosity
HRM : high-resolution melt
IM : interval map
InDel : insertion/deletion
IPGRI : International Plant Genetic Resources Institute
ISSR : inter-simple sequence repeat
kb : kilobase
KEGG : Kyoto Encyclopedia for Genes and Genomes
KOG : eukaryotic orthologous group
KW test : Kruskal-Wallis test
LDL : low-density lipoprotein
LG : linkage group
LOD : logarithm odd density
M : molar
MAS : marker-assisted selection
Mb : Megabase (million bases)
$MgCl_2$: magnesium chloride
min : minute
mL : milliliter
mM : millimolar

MQM : multiple QTL mapping
MTs : metric tons
MUFAs : monounsaturated fatty acids
$Na_2S_2O_5$: sodium metabisulfite
NaCl : sodium chloride
NCED3 : 9-cis-epoxycarotenoid dioxygenase-3
ng : nanogram
NGS : next-generation sequencing
NILs : near-isogenic lines
NR : non-redundant
nSSR : nuclear SSR
PCA : principal coordinate analysis
PCo : principal coordinates
PCR : polymerase chain reaction
PIC : polymorphic information content
PUFAs : polyunsaturated fatty acids
PVP : polyvinylpyrrolidone
qRT-PCR : quantitative real-time PCR
QTL : quantitative trait loci
RAD : restriction site-associated DNA
RAPD : random amplified polymorphic DNA
RE : restriction enzyme
RFLP : restriction fragment length polymorphism
RILs : recombinant inbred lines
rpm : revolutions per minute
s : second(s)
SAMPL : selectively amplified microsatellite polymorphic loci
SCAR : sequence-characterized amplified region

SNP : single nucleotide polymorphism
SOS1 : son of sevenless homolog-1
SPSS : statistical package for the social sciences
SRAP : sequence-related amplified polymorphism
SSR : simple sequence repeat
STS : sequence-tagged sites
T : Thymine
Taq : *Thermus aquaticus*
TBE : tris-boric acid/EDTA
TE : Tris-EDTA
TÜBİTAK : Scientific and Technological Research Council of Turkey
TÜİK : Turkish Statistical Institute
UCB-1 : University of California Berkeley-1
UPGMA : unweighted pair group method with arithmetic mean
UV : ultraviolet
λ : lambda
µL : microliter
µM : micro-molar
χ^2 : chi-square

1. INTRODUCTION

Pistachio (*Pistacia vera* L.) is highly commercially important and well-known nut trees that belong to the Anacardiaceae, a flowering plants group in the cashew family, (Kafkas et al., 2006a), which also includes cashew, mango, pepper tree, poison ivy, poison oak, and sumac. The genus, *Pistacia* consists of about 11 species that have an estimated age of around 80 million years (Parfitt and Badenes, 1997).

The pistachio is inhabited in the arid zones of Central Asia; it has a cultivation history for 3,000-4,000 years in Iran and later was introduced into Mediterranean Europe at the starting of the Christian era (Crane and Iwakiri, 1980). Pistachio cultivation was widening westward from its center of origin in Spain, Italy and other Mediterranean regions of Southern Europe, the Middle East, North Africa and to China and more recently to the United States and Australia (Hormaza et al., 1994a, 1998). *P. vera* is widely distributed, which ranged from the Kirgiz mountainous range to the Parapamiz and from the Boam canyon in the Kirgiz mountainous range to Southwest of the Kopetdag (Kayimov et al., 2001).

At present, Iran, the USA, Turkey, China, and Syria are known as the major pistachio producers. According to the latest report by the Food and Agricultural Organization of the United Nations (FAO), the total production of pistachio in the world reached 1,465,099 Metric Tons (MTs) in 2018. Iran was the highest producer in 2018 with a production of 551,307 MTs of pistachio nut followed by 447,700 MTs in the USA, 224,000 MTs in Turkey, 74,828 MTs in China, and 43,299 MTs in Syria. The quantity of nut production in 2016, 2017 and 2018 by major producing countries are given in Table 1.1 (Faostat, 2020).

Table 1.1. The production of pistachio nuts in 2016, 2017 and 2018 by five major producing countries.

2016			2017			2018		
Country	*MTs*[*]		*Country*	*MTs*[*]		*Country*	*MTs*[*]	
USA	406,646	Total production in the world was 1,256,848 MTs[*]	Iran	574,987	Total production in the world was 1,210,360 MTs[*]	Iran	551,307	Total production in the world was 1,465,099 MTs[*]
Iran	405,925		USA	272,291		USA	447,700	
Turkey	170,000		China	95,294		Turkey	240,000	
China	90,082		Turkey	78,000		China	74,828	
Syria	57,910		Syria	56,508		Syria	43,299	

[*]MTs = Metric Tons

According to FAO, the total harvested land areas in the world reached 1,180,365 hectares (ha) in 2018. Iran was the highest harvested country of pistachio in 2018 with a total harvesting land area of 825,286 ha followed by 106,837 ha in the USA, 70,087 ha in Turkey, 50,122 ha in Syria, and 29,393 ha in Spain. The harvested area of pistachio by major harvesting countries is shown in Table 1.2 (Faostat, 2020).

Table 1.2. The harvested area of pistachio in 2016, 2017 and 2018 by five major harvesting countries.

2016			2017			2018		
Country	*ha*[*]		*Country*	*ha*[*]		*Country*	*ha*[*]	
Iran	378,181	Total harvested area in the world was 722,569 ha[*]	Iran	429,535	Total harvested area in the world was 800,940 ha[*]	Iran	825,286	Total harvested area in the world was 1,180,365 ha[*]
USA	96,720		USA	101,171		USA	106,837	
Syria	61,716		Turkey	68,237		Turkey	70,087	
Turkey	60,814		Syria	64,789		Syria	50,122	
China	29,188		Tunisia	31,980		Spain	29,393	

[*]ha = Hectare

According to the Turkish Statistical Institute (TÜİK), Şanlıurfa and Gaziantep are the main producing provinces in Turkey with a production quantity of 28,967.32 MTs and 23,897.97 MTs respectively in 2019. Followed by Şanlıurfa and Gaziantep, three other provinces, namely Adıyaman, Siirt, and Kahramanmaraş

produced 2,419.46 MTs, 11,074.91 MTs, and 918.07 MTs, respectively in 2019. The quantity of pistachio nut production in 2015, 2016, 2017, 2018 and 2019 by major producing provinces of Turkey are presented in Table 1.4 (Tüik, 2020).

Table 1.3. The production of pistachio nuts in 2015, 2016, 2017, 2018 and 2019 by major producing provinces in Turkey.

Province	*2015 (in MTs*)*	*2016 (in MTs*)*	*2017 (in MTs*)*	*2018 (in MTs*)*	*2019 (in MTs*)*
Şanlıurfa	43,406.99	43,641.09	25,861.18	90,815.87	28,967.32
Gaziantep	48,179.69	68,309.29	13,391.89	81,812.94	23,897.97
Adıyaman	13,941.62	17,016.99	9,471.03	21,786.12	2,419.46
Siirt	10,179.52	6,089.94	7,206.69	10,252.13	11,074.91
Kahramanmaraş	1,993.09	5,555.61	3,360.22	3,586.11	918.07

*MTs = Metric Tons

The pistachio tree is small to medium in size that is bushy and deciduous. It grows very slowly up to a stature of about 6–9 meters with an individual to multiple trunks. The leaves of pistachio tree are compound-pinnate, and hairy when in the juvenile stage, and glabrous when it become old that have three to five elliptical leaflets. Pistachio is a dioecious tree. The staminate and pistillate inflorescences are panicles assembled in the axils of the formerly year's growth. It consists of a few hundred single flowers (Crane and Iwakiri, 1981). Both categories of flowers are apetalous that is pollinated by wind. Because of mature pistillate flowers have zero relics of stamens, *P. vera* shows perfect dioecy (Wannan and Quinn, 1991). Nonetheless, the initiation of stamen and carpel primordia occurred in both male and female types of flowers. But in the primordial stage, the development of organs of the reversed sex becomes seized (Hormaza and Polito, 1996). There are ranges from two to five tepals and a pistil with three stigmas in most of the matured flowers. The commercially important pistachios that are familiar for its fleshy exocarp and mesocarp or hull that has hard in form, dehiscent and bony endocarp or shell. It splits length-wise when the fruit has matured (Hormaza and Wunsch, 2007).

The suitable growth latitude of pistachio is at 36-37° latitude. The pistachio as a deciduous tree and drought resistant. Pistachio is tolerant of hot and dry summer weather. Cold weather is necessary to satisfy the chilling requirements. However, hot and dry summers are required to produce nuts (Ak, 2014).

The dioecy character of pistachio has been recognized since 1697 (Whitehouse and Stone, 1941). Pistachio flower lacks petals which eventually fails to attract insect pollinators. And thus, pollination takes place by transporting of pollens from male to female trees by the wind. Thus, the pistachio orchards must have both male and female trees. The ratio for male and female trees should be 1/8 or 1/11 (Ayfer, 1964). In most of the pistachio orchards in Turkey, the first two weeks of April is observed as the flowering time. To ensure maximum nut production, enough numbers of male and female trees should be present in the orchard to ensure adequate pollination (Ak, 2014).

P. vera is the only cultivated species in the Anacardiaceae family due to having its edible nuts that have beneficial properties to human health as well as commercial importance in sweetmeat industries. On the other hand, the wild *Pistacia* species have edible small seeds that are also used for a suitable source of rootstock for cultivated *P. vera* (Kafkas et al., 2002). Some other *Pistacia* species are used in soap and oil industries by many local inhabitants (Hepper, 1992).

Pistachio is attributed to the “green gold tree” for its huge commercial and nutritional values (Benmahioul et al., 2012). Some archaeological evidence in Turkey indicates the pistachio nuts were being used as food in 7,000 B.C. (Kaska, 1990). Pistachio has a high nutritional value due to its high protein, vitamin, mineral, and unsaturated fat content. Pistachio kernels accommodate both monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The major unsaturated fatty acids found in pistachio oil are oleic, linoleic and linolenic acids (Acar et al., 2008). The unsaturated fatty acids are a nutritional provision due to their health-related advantages. Essential fatty acids are decisive for the human diet for

the preservation of growth and reproduction. Linoleic acid, which is an essential fatty acid of the omega-3 group, is highly crucial for the growth and maintenance of the nervous system and physiological activities in humans because of its reduction property in total and LDL (low-density lipoprotein) cholesterol levels (Sari et al., 2010). According to Kris-Etherton et al. (1999), MUFAs, such as oleic acid, have properties in decreasing plasma triacylglycerol and cholesterol concentrations. Similarly, PUFAs, such as linoleic and linolenic acids, assist in preventing cancer, atherosclerosis, heart disease, and diabetes (Chahoud et al., 2004). The oils with lower linoleic and linolenic acid contents usually have an extended shelf life, and monounsaturated fatty acids are highly enticing due to their well-being benefits (Savage et al., 1999). Pistachio shows nutritional advantages in decreasing blood cholesterol. A 20% of the consumption of pistachio of daily-basis caloric intake edges to remarkable improvement in HDL (high-density lipoprotein) and inhibits LDL cholesterol oxidation. Pistachio is beneficial for prevention as well as the treatment of coronary artery disease. This is due to having the high levels of both MUFAs and PUFAs, and probably for the tocopherol content (Acar et al., 2017).

Pistachio is a diploid tree having a haploid chromosome (n = 15) that also has a higher degree of heterozygosity. Thus, pistachio is considered as a model for genetic linkage mapping studies in which both inter- and intra-specific hybrids are possible to be easily produced (Kafkas et al., 2003). F_1 progenies obtained from the intercross between two heterozygous ancestors are typically used for genetic mapping studies in outbreeding heterozygous tree species. Therefore, by analyzing the markers and the double pseudo-testcross strategy, linkage maps can be constructed (Grattapaglia and Sederoff, 1994).

There is a much bit of cytological data available for *Pistacia* species within the published literature. The foremost study *P. vera*'s chromosome number (2n = 30) was reported by Zohary (1952). Three *Pistacia* species: *P. lentiscus* with 2n = 24, *P. atlantica* with 2n = 28 and *P. vera* with 2n = 30 was used to reveal the chromosome

number. Harandi et al. (1996), and Harandi and Behboodi (1997) also studied to counts the chromosome number in *Pistacia* species. They reported the chromosome numbers in *P. vera* as 2n = 30, *P. khinjuk* as 2n = 24 and the other subspecies of *P. atlantica* as 2n = 28. After publishing the results of the above studies, other researchers, since then, also reported the same chromosome numbers for *P. vera* (Maggs, 1973; Ozbek, 1978).

Pistachio is a perennial plant with a long juvenile duration of almost 5-10 years. *Pistacia* species are usually dioecious, however, monoecious species in *P. atlantica* were reported (Kafkas et al., 2000). Dioecy character and long juvenile period are main constraints encountered during breeding and in genetic applications in *Pistacia*. These characteristics eventually cause large investment of time, labor, and land for the evaluation and characterization of pistachio individuals in a breeding experiment. Moreover, it is not clearly understood the genetic regulation of the most economically important traits that included disease along with pest control, nut quality, and sex determination system in *Pistacia* (Turkeli and Kafkas, 2013). Thus, molecular markers are a favorable choice to mitigate this kind of barrier in the pistachio breeding programs.

In plant breeding, a genetic marker is defined as an allelic form of genes or genetic loci that determines the biological structure. Genes can be transmitted among generations, and therefore, can be utilized as experimental probes to identify a particular individual or a tissue, a cell, a nucleus, a chromosome or even a gene. Genetic markers could be categorized broadly into two major classes, namely, classical markers, and DNA (deoxyribonucleic acid) markers (also known as molecular markers) that are used in breeding programs. Classical markers are subclassified as morphological, cytological, and biochemical markers, whereas, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), single nucleotide

polymorphism (SNP), and Diversity Array Technology (DArT) marker are some examples of molecular markers (Collard et al., 2005; Jiang et al., 2013).

Until the advancement of molecular marker technology, classical markers were broadly used in plant breeding. Morphological markers are visibly distinguishing qualities like seed structure, the color of flower, growth manner, and other crucial agronomic traits. They are easy for utilizing with no requirements for specific appliances, however, the use of morphological markers is limited for its few numbers and it is influenced by stages of plant growth and some other environmental aspects (Eagles et al., 2001). Markers related to the variations in numbers, the pattern of banding, size, shape, order, and position of chromosomes to be known as cytological markers. They are utilized in both differentiation of original and mutated chromosomes, and to identifying linkage groups, and to the physical mapping (Jiang et al., 2013). Biochemical markers, also known as isozymes are multi-molecular fashions of enzymes, coded by various genes and having identical functions. These markers can be applied in the identification of hereditary assorted variety, population structure, the flow of genes, and sub-division of population. This marker system is codominant, simple to utilize, and financially savvy, be that as it may, they are less in number, they recognize less polymorphism, and they are influenced by different extraction procedures, plant tissues, and distinctive plant development stages (Bayley, 1983; Mateu-Andres and De Paco, 2005; Mondini et al., 2009).

With the improvement of molecular marker innovation, thinking back to the 1980s, the destiny of plant breeding has been changed. Molecular markers are nucleotide sequences (patterns) and can be researched through the polymorphism present within the nucleotide sequences of various individuals. Insertion (addition), deletion (depletion), duplication, point mutation, and translocation is the premise of these polymorphisms, in any case, they do not influence the activity of genes (Mondini et al., 2009). When selecting an ideal molecular marker, the selection criteria should be included: (1) high level of polymorphism, (2) even distribution

throughout the genome, (3) codominance in nature that allows distinguishing heterozygosity, (4) distinct allelic features, (5) no pleiotropic effect, (6) cost-effective for marker development and genotyping, (7) easy detection and computation/automation, (8) great availability and suitability, and (9) no harmful effects on phenotype (Jiang et al., 2013).

Molecular markers can be grouped in a few different ways depending on their method of gene activity (co-dominant or dominant markers), approach for recognition (hybridization-based molecular markers or polymerase chain reaction (PCR)-based markers), and method of transmission (parental organelle inheritance, maternal organelle inheritance, bi-parental molecular inheritance or maternal nuclear inheritance) (Semagn et al., 2006).

RFLP was the foremost and only molecular marker technique based on hybridization. Polymorphism occurs as a result of insertion/deletion (InDel), point mutation, translocation, duplication and inversion among the individuals from the same species. RFLP is a co-dominant marker system with high reproducibility. RFLP is present on the genome at a high rate. However, RFLP shows a medium level of polymorphism and it requires high quality and quantity of extracted DNA to be identified. Moreover, it is an expensive method (Nadeem et al., 2018).

PCR-based techniques include AFLP, RAPD, SSR (also known as microsatellite DNA), and ISSR markers. AFLP is a dominant marker system with an intermediate level of reproducibility, having high polymorphisms is richly abundant in the genome. It requires a low quantity of DNA to be identified. However, the high establishment cost sometimes limits the use of it. RAPD is a dominant marker system, which is very highly polymorphic and is highly reproducible. The establishment cost of the RAPD marker system is very low. It is very highly abundant in the genome. However, its dominance in nature limits the use in some studies. SSR is a co-dominant marker system that has a high polymorphism level and is highly reproducible. It requires low DNA quantity to be identified. It is

available in the genome at a medium level. However, a high establishment cost sometimes limits the use of this marker system. ISSR is a dominant marker system with medium to high reproducibility that has a high polymorphism level. A low quantity of DNA is required to identify the ISSR markers. Medium genomic abundance along with high establishment cost are the main constraints to use this marker system. DArT is a dominant marker system with a high polymorphism level and high reproducibility. The genomic abundance of DArT markers is very high. The establishment cost is very cheap. A low quantity of DNA is required to identify the DArT markers in the genome. However, its dominant nature sometimes limits the use of it (Nadeem et al., 2018).

An SNP is defined as a solitary nucleotide base contrast between two DNA sequences or individual. SNPs can be sorted by nucleotide substitutions either as transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G). In conduct, single-base variations in cDNA (complementary DNA) are considered as SNPs as are individual base InDel in the genome. The SNPs give the least difficult type of molecular markers as an individual nucleotide base is the tiniest unit of inheritance, and in this way, they can give the highest number of markers. SNPs can be found ordinarily in plants. Typically, SNP frequencies are in the scope of one SNP each 100-300 base pairs (bp) in plants. SNPs may exhibit inside coding arrangements of genes, non-coding regions of genes or in the intergenic areas between genes at various frequencies in various chromosomal regions (Edwards et al., 2007).

SNPs are co-dominant markers, regularly connected to genes and are found in the simplest type of polymorphism, and in this manner, they become exceptionally appealing and important genetic markers in the hereditary study and plant breeding. Besides, SNPs can be handily automated and immediately identified, with high productivity for the recognition of polymorphism. Along these lines, it tends to be extraordinary that SNPs will be progressively utilized for different purposes, especially as whole DNA sequencing becomes accessible for an ever-increasing

number of species (e.g., rice, soybean, maize, and so forth). In any case, significant expenses for a start-up for marker development, the requirement of high-quality DNA and high technical demand in some extent limit, the utilization of SNPs in certain research centers and practical breeding programs (Rafalski, 2002; Duran et al., 2009; Edwards and Batley, 2010).

A rapid discovery method for SNP, known as DArT-Seq™, was advanced based on the DArT marker platform with a mix of next-generation sequencing platforms (Cruz et al., 2013; Raman et al., 2014). DArT-Seq™ represents new employment of the sequencing of complexity-reduced portrayals. Because it is optimized for each organism by selecting the most appropriate reduction method. It gained popularity rapidly as a GBS (genotyping-by-sequencing) method (Altshuler et al., 2000) with more recent applications within the same concept on the next-generation sequencing (Elshire et al., 2011). DArT-Seq™ had applied successfully in many species. In a standard DArT assay, approximately 200,000 genomic fragments are sequenced 10 times, and most of the samples experiment in duplicate to make sure the reproducibility. After very strict filtering processes DArT-Seq™ offers cost-effective genome profiling through the production of high-density SNPs and silico DArT (presence/absence of restriction fragments in representation) markers (Killian et al., 2012; Cruz et al., 2013; Raman et al., 2014).

Several molecular marker systems such as RAPD (Hormaza et al., 1994b; Kafkas and Perl Treves, 2001; Kafkas et al., 2001), SSR (Zaloglu et al., 2015), SRAP (sequence-related amplified polymorphism; Ahmad et al., 2005) AFLP (Kafkas, 2006), ISSR (Kafkas et al., 2006b), SAMPL (selectively amplified microsatellite polymorphic loci; Karimi and Kafkas, 2011), and SNP (Kafkas et al., 2015) have been employed to assess the genetic diversity, fingerprinting, germplasm characterization, phylogenetic studies, genetic linkage mapping, and sex determination, both in cultivated and wild *Pistacia* species.

Genetic mapping, which is also referred to as linkage or meiotic mapping uses to the determination of relative position and distances among markers in a chromosome. Genetic linkage mapping is based on the principle that genes or markers or loci segregate via chromosomal recombination at the time of the meiosis phase of cell division. Distance between two markers on a linkage map defined as the mean number of recombination events on a given chromatid, in that region per meiosis. A molecular marker is a precursor to construct genetic linkage maps. Genetic linkage maps reveal valuable information about the segregation and the genetic architecture of a species. A linkage map is constructed by analyzing the recombination frequency of markers. This obtained information can be used as a basis for determining the relative locations of these markers in the genome. To construct a linkage map of a plant species, a suitable population with adequate sample size is required. According to the scientific literature on molecular breeding, a sample size of 50-1,000 from F_1, F_2, BC (back cross), RILs (recombinant inbred lines), DHLs (doubled haploid lines), or NILs (near-isogenic lines) population are suitable for genetic mapping studies (Semagn et al., 2006).

Since a high-density genetic linkage map is still to be constructed using the SNP marker system and a few studies have been performed in QTL analysis that is not sufficient to understand the inheritance of several traits in pistachio. Therefore, in the scope of this current study, we aimed:

(i) to construct high-density genetic linkage maps using SNP marker system, and

(ii) to perform QTL analysis of leaf, shoot, and flowering traits in an interspecific F_1 population in pistachio.

2. LITERATURE REVIEWS

2.1. Studies on Molecular Marker Development and Its Uses in *Pistacia*

The first DNA marker study was published by Hormaza et al. (1994a), who studied the genetic diversity and relationships among 15 *P. vera* cultivars by using RAPD markers. They utilized the subsequent information to develop a similarity matrix and to play out a UPGMA (unweighted pair group method with arithmetic mean) cluster analysis. They recognized two significant groups of *P. vera* germplasms, in particular, the Mediterranean cluster that incorporates cultivars beginning from the Mediterranean regions of Europe, North Africa, and the Middle East; and an Iranian-Caspian cluster that incorporate germplasm starting from the Zagros mountain zones in addition to 'Peters', a cultivar of obscure source in the USA. Their investigation underpins the speculation that pistachio development began in or close to the Mediterranean locale of the Middle East.

Hormaza et al. (1994b) again published their works where they found sex-linked RAPD markers helping sex determination in *P. vera*. They utilized 700 diverse decamer oligonucleotide primers to perform DNA amplification in which one (OPO08) created a 945 bp amplification band that was available just in the mass female samples and missing in the bulk male samples. They suggested that the band was firmly connected to the gene(s) that control sex identification in pistachio. The $OPO08_{945}$ RAPD marker could be utilized in breeding projects to screen the sex of pistachio. To confirm the suspicion, they screened 94 extra seedlings with the OPO08 primer and got results predictable with a 1:1 male: female proportion.

Hormaza et al. (1998) considered the example of relatedness among 29 *P. vera* cultivars and accessions utilizing the RAPD marker system. The outcomes demonstrated a significant level of polymorphism in the species accentuating the significance of safeguarding the staying wild stands of *P. vera*. The investigations upheld the idea that cultivars being used west of the Zagros-Caucasus go likely

started from a constrained germplasm base. The new outcomes demonstrated more noteworthy hereditary diversity, reliable with the theory that pistachio development started in or close to South Central Asia.

Parfitt and Badenes (1997) utilized PCR enhancement followed by restriction scrutiny of a 3.2-kilobase (kb) region of variable chloroplast DNA (cpDNA), and RFLP investigation of the *Pistacia* cpDNA with tobacco cpDNA tests to contemplate the phylogenetic connections of 10 *Pistacia* species. They utilized both parsimony and cluster analysis to partition the family into two significant groups. *P. vera* was resolved to be the most antiquated of the 10 species. *P. integrrima* and *P. chinensis* were demonstrated to be distinct species. In their examination, a developmental pattern from huge to little nuts and leaves with not many, enormous pamphlets to many, little handouts were bolstered.

Kafkas and Perl-Treves (2001) contemplated the ordered connections and hereditary deviation of wild *Pistacia* germplasm in Turkey utilizing morphological information and RAPD examination. They tested and portrayed a sum of 40 wild *Pistacia* genotypes from various regions of Turkey that included 10 *P. eurycarpa* (locally distinguished as *P. khinjuk*), 20 *P. atlantica*, and 10 *P. terebinthus* genotypes. They likewise included two nearby *P. vera* varieties for the correlation in their examination. They performed cluster analysis dependent on morphological information uncovered that the *P. vera* and *P. eurycrpa* are the nearest species followed by *P. atlantica* and *P. terebinthus*. They utilized 10 polymorphic RAPD primers that yielded an aggregate of 138 scorable bands for DNA fingerprinting of these genotypes. In the case of molecular phylogeny, four *Pistacia* species were unmistakably isolated from one another. *P. terebenthus* gave off an impression of being the most separated species, and the nearest pair of species was seen as *P. atlantica* and *P. eurycarpa*. These outcomes upheld the grouping of trees that were recognized by local producers as *P. khinjuk*, identified as *P. eurycarpa* in their investigation.

Kafkas et al. (2001) looked for RAPD markers connected to sex in *P. atlantica*, *P. terebinthus* and *P. eurycarpa* that are the fundamental wild species in Turkey that are utilized as rootstock for cultivated *P. vera*. They screened an aggregate of 472 primer pairs that were intensified by two primers, in particular, BC156 and BC360 to be related to sex in *P. eurycarpa*. They examined the acquired bands in 30 male and 37 female individuals. The $BC156_{(1300)}$ band was available in all, except for one, female trees and was missing in the entirety of the male trees. The $BC360_{(500)}$ band was enhanced in 31 out of 37 females and was missing in all-male trees. In *P. atlantica*, the primer, OPAK09 intensified a female-related band (850 bp) that was available in each of the 46 female individuals examined and missing in every one of the 38 male individuals examined. They accepted these markers are connected to sex-determining loci.

Ahmad et al. (2003) considered the improvement of microsatellite motifs from 'Kerman' pistachio (*P. vera*) by building up a library improved with dinucleotide $(CT)_n$ and $(CA)_n$, and trinucleotide $(CTT)_n$. In the $(CT)_n$ enhanced library, compound sequences were 45% while for $(CA)_n$ it was 13.5%. In both dinucleotide enhanced libraries, about 80% of the clones having a microsatellite had a recurrent length of 10 to 30 units. A library advanced for trinucleotide $(CTT)_n$ contained <19% of the clones with $(CTT)_n$ repeats. Clones that contained microsatellites, 62% had adequate flanking sequences for primer design. At first, 25 primer sets were planned in which 14 primer sets were intensified plainly and created an effectively interpretable PCR product in the economically significant American, Iranian, Turkish, and Syrian pistachio cultivars. In all-out 14 alleles were distinguished by 14 primer sets and a dendrogram was developed based on the data. The SSR markers recognized a large portion of the examined cultivars from their unique DNA fingerprint. A UPGMA cluster investigation indicated the vast majority of the Iranian samples in a single group while the Syrian samples were the most

different and did not comprise a single particular group. The scientists found the most extreme number of cultivar-explicit markers in 'Kerman'.

Ahmad et al. (2005) utilized SSR and SRAP marker frameworks to portray four economically significant pistachio rootstocks: two types of *P. atlantica* cv. 'Standard Atlantica', *P. integerrima* cv. 'Pioneer Gold' and two interspecific hybrids of the equivalent, 'Pioneer Gold II' and 'UCB-1 (University of California Berkeley-1)'. An aggregate of 35 putative alleles was identified by 12 SSR primer sets with a normal of 2.9 alleles per locus. The number of putative alleles extended from 2 to 5 in the tried pistachio rootstock. The number of groups delivered by SRAP markers was exceptionally variable, running from 11 to 38, with a normal of 25.2 per primer combination. Eight primer sets brought about 104 (51%) polymorphic markers in these samples. SSR and SRAP markers were distinguished all pistachio rootstocks examined from their remarkable fingerprints. Both SSR and SRAP markers affirmed that the watched variety in 'UCB-1' rootstock was hereditary. The authors likewise found the proof of contaminating pollen other than from *P. integerrima* in some hybrids 'UCB-1' rootstock offspring delivered by crossed pollination. They saw that only alleles from the cultivar 'Standard Atlantica' in strange 'UCB-1' rootstock in the nursery. They likewise found that the terrible showing of the scion cv. 'Kerman' on 'UCB-1' rootstock was not due to 'UCB-1' rootstock showing unusual conduct in the nursery.

Yakubov et al. (2005) built up a female DNA marker in *P. vera* utilizing combined sequence characterized amplified regions (SCAR) primers in the touchdown PCR technique. The preliminary $OPO08_{945}$ enhanced as a 905 bp section taking all things together female trees, yet besides in a few male trees. SCAR primers were designed dependent on the RAPD female markers and intensified a 905 bp female and 909 bp male fragments, which checked on 54 distinctive pistachio genotypes. Based on a polymorphic locus, a second inward arrangement of SCAR primers was structured, in blend with a touchdown PCR method. All females

enhanced a particular 297 bp product, while females had no amplified item. Therefore, a new set of SCAR primer pairs with touchdown PCR were suggested to determine females in a breeding program or a germplasm collection in *P. vera*.

Kafkas et al. (2006a) contemplated molecular characterization of 69 pistachio cultivars and genotypes collected from seven countries utilizing RAPD, ISSR and AFLP markers. The outcomes demonstrated that every one of the three marker systems had the option to uncover the changeability among the tested cultivars and genotypes. The relationship between RAPD and AFLP was the most elevated (r = 0.73) and the incentive among RAPD and ISSR was the least (r = 0.58). AFLP ends up being the best method among them. ISSR and AFLP markers were solid and delivered reproducible bands. ISSR was favored over RAPD. The built UPGMA dendrogram acquired from the joined information isolated the tested genotypes into two fundamental groups: Iranian and Mediterranean. The Mediterranean group additionally separated into three subgroups: 'Siirt', 'Turkish' and 'Syrian'. The closeness of the groups was 'Iranian' – 'Siirt' – 'Turkish'/'Syrian'. The authors inferred that these discoveries uncover another comprehension of the relocation of pistachio cultivation from its focal point of beginning, the Iranian-Caspian locale, utilizing Southeastern Turkey to Syria, the Mediterranean region of Europe, and Northern Africa.

Kafkas et al. (2006b) portrayed 17 wild Afghan pistachio accessions and three cultivated genotypes utilizing the AFLP marker framework. They created an aggregate of 288 AFLP fragments utilizing eight AFLP primer sets. The number of enhanced fragments shifted from 18 to 48 for every primer pair and 136 bands were polymorphic, with an average of 17 polymorphic groups for each primer pair. The polymorphic groups ran from 26.2% to 79.2% per primer pair. Given the UPGMA clustering of the Nei and Li distance matrix, the 17 wild accessions were grouped by the region of origin and were particular from the three cultivars.

Kafkas (2006) examined the phylogenetic connections among 31 wild *Pistacia* accessions utilizing AFLP markers. Six AFLP primer pairs produced a sum of 275 fragments with an average of 45.8 bands per primer pair, of which 254 (45.8) were polymorphic. The author performed UPGMA and PCo (principal coordinates) analysis utilizing both mean character difference and Jaccard similarity matrix to portray the relationships. The resulted cluster was isolated into two groups. *P. vera*, *P. khinjuk*, *P. eurycarpa*, *P. atlantica*, *P. mutica*, *P. integerrima*, and *P. atlantica* x *P. integerrima* hybrids occurred in the first cluster. The subsequent cluster comprised of *P. palaestina*, *P. terebinthus*, *P. mexicana*, and *P. lentiscus*. The *P. khinjuk* accession had nearer connections to *P. eurycarpa* and *P. atlantica* than to *P. vera*, which prompted the misidentification of *P. khinjuk* samples as *P. eurycarpa* in their investigations. The author revealed *P. atlantica – P. mutica* and *P. terebinthus – P. palaestina* matches as the nearest species, and in this way, they grouped *P. mutica* as *P. atlantica* and *P. palaestina* as *P. terebinthus*. The author claimed that the resulted dendrogram and the PCo plots did not bolster the evergreen versus deciduous sectional division of *Pistacia* species and proposed classifying in a separate cluster instead of being in the first cluster.

Mirzaei et al. (2006) contemplated the phylogenetic connections among wild pistachio species utilizing RAPD markers that included *P. vera* var. 'Sarakhs', *P. atlantica* subsp. *mutica* ('Baneh'), *P. khinjuk* and six cultivars of *P. vera* including 'Khanjary Damghan', 'Ohadi', 'Ghazvini Zoodras', 'Akbary', 'Nishkalaghi', and 'Italiaei Zoodras'. They examined 77 primer sets of which 11 primer sets were chosen that demonstrated polymorphism in the tested genotypes. This primer sets uncovered 79 polymorphic fragments. The authors utilized Jaccard's similarity coefficient and UPGMA algorithm for cluster analysis. The observed pistachio genotypes were isolated into four distinct groups. The outcome put *P. vera* var. 'Sarakhs' between the wild and cultivated pistachio species. The researchers claimed that the outcomes from their examination reinforce the hypothesis that all pistachio

cultivars were originated from *P. vera* var. 'Sarakhs'. In their resulted outcomes, *P. vera* var. 'Sarakhs' demonstrated the most association with pistachio cultivars while *P. khinjuk* and *P. atlantica* subsp. *mutica* demonstrated the least relationship.

Basha et al. (2007) contemplated the hereditary assorted variety of Syrian pistachio assortments utilizing AFLP markers. They gathered 114 accessions from 37 unique ranches to evaluate decent variety at morphological and molecular levels. Molecular characterization was completed by the AFLP technique. They utilized seven AFLP primer sets for their investigation. They distinguished 25 female assortments of pistachio in Syria, some of which were unique and described for the first time. They recognized three distinct groups of diversity by cluster analysis which gives helpful data about the distribution of genetic diversity in Syria for improved use of sustainable conservation.

Shanjani et al. (2009) examined the molecular variation between and within the cultivated and wild *Pistacia* species utilizing AFLPs. They utilized 216 pistachio accessions in their examination that included seven populations from three wild species viz. *P. vera*, *P. khinjuk*, and *P. atlantica* subsp. *kurdica*. The cultivars were gathered from Iran with some minor foreign cultivars. The Iranian cultivars indicated a significant level of genetic diversity alongside a clear and distinct partition of foreign cultivars anticipated by UPGMA and arithmetic averaging. The least polymorphism was seen in *P. atlantica* subsp. *kurdica* alongside the most reduced number of complete groups contrasted with different species. The acquired outcomes uncovered a solid genetic disintegration of *P. atlantica* subsp. *kurdica* that mirrored a serious decrease in inhabit and over-exploitation.

Baghizadeh et al. (2010) considered the genetic diversity of some Iranian pistachio cultivars utilizing RAPD, ISSR, and SSR marker strategies. They utilized 31 pistachio cultivars and genotypes in their examination. They developed a general dendrogram utilizing the combined data of three sets of molecular markers. The general principal coordinate analysis (PCA) in light of the genetic similarity matrix

indicated that the initial three eigenvectors represented 28.46% of the total molecular variation. Therefore, the PCA results approved and affirmed the aftereffects of cluster analysis. While utilizing SSR primers, the four primers created 11 alleles among 31 tested pistachio genotypes with an average of 2.75 alleles. They watched a 100% polymorphism at all of the loci. The low average polymorphic information content (PIC) estimation of 0.4374 showed that the nearness of high genetic similarity among genotypes and entails the improvement of extra polymorphic SSR primers for the progressively successful portrayal of Iranian pistachio cultivars and genotypes. In their examination, as indicated by the effective multiplex ratio and assay efficiency index, RAPD markers had been appeared as the most remarkable markers to separate the genotypes followed by ISSR and SSR markers, respectively.

Pazouki et al. (2010) contemplated the hereditary assorted variety and connections among *Pistacia* species and cultivars. The analysts utilized 282 Iranian pistachio genotypes (*Pistacia* spp.) together with 22 foreign cultivars of *P. vera*. The samples were genotyped utilizing 10 SSR marker sets. The outcomes indicated that the hereditary assorted variety inside *P. atlantica* subsp. *kurdica* was extensively not exactly in *P. vera* and *P. khinjuk*. PCo examination uncovered an unmistakable partition between the distinctive *Pistacia* species just as between the Iranian and foreign cultivars. Analysis of molecular variance (AMOVA) indicated that the variety between the species, between various populations, and within populations represented 41%, 9%, and 50% of the total variation, respectively. The outcomes exhibited that the utilization of the SSR marker strategy gives significant criticism in the investigation of hereditary assorted variety and connections among *Pistacia* species and cultivars.

Arabnezhad et al. (2011) examined the hereditary connections among Iranian pistachio genotypes utilizing the SSR marker created from *P. khinjuk* Stocks. The authors created two DNA libraries enriched for dinucleotide $(AG)_n$ and trinucleotide $(ATG)_n$ microsatellite motifs from the *P. khinjuk* genome. By utilizing

the colony PCR strategy, they sequenced 44 clones and structured 27 sets of primers from flanking regions of the repeats. While inspected the primer sets, it demonstrated effective cross-species enhancement within the genus *Pistacia*. They developed a dendrogram based on the minimum evolution clustering algorithm, uncovered that *P. vera* had a closer association with *P. khinjuk* than with *P. integerrima*, *P. palaestina*, *P. atlantica*, and *P. mutica*. The dendrogram further separated the wild 'Sarakhs' pistachio from the remainder of the pistachio genotypes recommending that the tested genotypes of *P. vera* are evolved from 'Sarakhs' wild variety. Along these lines, the authors inferred that the wild 'Sarakhs' pistachio played a pivotal role in the evolution of domesticated pistachios in Iran.

Karimi and Kafkas (2011) contemplated the hereditary relationships among *Pistacia* species including *P. vera*, *P. khinjuk*, *P. eurycarpa*, *P. atlantica* subsp. *atlantica*, *P. atlantica* subsp. *mutica*, and *P. atlantica* subsp. *cabulica* by utilizing the SAMPL marker system. Six primer sets delivered a sum of 182 bands, with an average of 30.33 bands per primer pair, of which 128 were polymorphic. The scientists built a UPGMA dendrogram that had three distinct branches; the primary branch contained *P. vera*, the subsequent branch contained *P. khinjuk*, *P. eurycarpa*, *P. atlantica*, and subsp. *mutica* and *cabulica*, while in the third branch a various leaflet was grouped. The UPGMA investigation likewise isolated *P. atlantica* subspecies from *P. eurycarpa*.

Karimi et al. (2011) considered the hereditary relationship among pistachio species utilizing AFLP markers. The analysts utilized an aggregate of 44 accessions belonging to *P. vera*, *P. eurycarpa*, *P. khinjuk*, all subspecies of *P. atlantica* (*atlantica*, *mutica*, *kurdica*, and *cabulica*), three obscure genotypes, and three accessions proposed to be hybrids from *P. eurycarpa* x *P. atlantica* in their examination. Six AFLP primer sets were utilized in their investigation that delivered an aggregate of 475 fragments, with an average of 79.16 fragments per primer pair, of which 336 groups were seen as polymorphic. To build a dendrogram, the analysts

utilized the UPGMA test on Jaccard's similarity coefficient matrix alongside the average similarity of every species. Given the outcomes acquired, two primary clusters were framed; the main group contained *P. vera*, *P. eurycarpa*, *P. atlantica* (subsp. *atlantica*, *kurdica*, *mutica*, and *cabulica*) alongside the hybrid genotypes, though the subsequent cluster included *P. khinjuk* alongside the accessions. The hybrid accessions were situated among *P. eurycarpa* and *P. atlantica* species. One of the obscure accession clustered with the hybrid ones and two others were assembled closely to *P. khinjuk*. Because of the outcomes got right now, the nearest species to *P. vera* was *P. eurycarpa*, trailed by *P. atlantica*. It appeared that UPGMA test isolated *P. atlantica* subsp. *mutica* and *cabulica* from *P. eurycarpa*. Subspecies *mutica* and *cabulica* were two nearest genotypes, consequently *P. atlantica* subsp. *mutica* could be delegated a particular variety as *P. mutica* and *cabulica* as a subspecies of *P. mutica*. Taking everything into account, the analysts asserted the examination uncovered that *P. eurycarpa* is an equivalent word of *P. atlantica* subsp. *kurdica* and ought to be viewed as of particular from *P. atlantica*. Be that as it may, *P. atlantica* demonstrated a closer hereditary closeness to *P. eurycarpa* than other species.

Esfandiyari et al. (2012) utilized *P. atlantica* subsp. *mutica*, *P. khinjuk*, and *P. vera* subsp. *sarakhs* to discover sex markers for wild species. Leaves were gathered from both male and female plants of every species for DNA extraction and their band patterns were investigated dependent on the attendance or nonattendance of explicit bands. Thirty RAPD primers and one set of SCAR primers were tried to distinguish a marker firmly connected to the sex loci in wild *Pistacia* species. Among the RAPD primers, just BC_{1200} was effectively enhanced a particular sex band present in female plants. Among every single individual sample, a fragment of around 300 bp was intensified in female trees however not in male ones. Even though the sex assurance mechanism in *Pistacia* is as yet obscure, they might be activated by a solitary locus. The scientists inferred that the SCAR marker strategy had ended

up being a solid procedure in the sexual orientation determination of pistachio genotypes at the seedling phenophase.

Talebi et al. (2012) contemplated molecular diversity variety and phylogenetic connections of *P. vera*, *P. atlantica* subsp. *mutica*, and *P. khinjuk* utilizing SRAP markers. The scientists utilized 36 pistachio accessions from Iran, Syria, Turkey, and the USA by screening 30 primer sets. Among them, 11 primer sets enhanced an aggregate of 202 fragments, of which 168 (83%) were polymorphic, with an average of 15.27 pieces for every primer pair. The cluster investigation demonstrated species-based grouping and the nearest types of *P. vera* is seen as *P. atlantica* followed by *P. kninjuk*. The acquired consequences of AMOVA examination showed that the greater part of the all-out molecular variance was inferable from disparity among the promotions from various species. Distinctive *P. vera* increases with wild 'Sarakhs' accession clustered in a different group dependent on SRAP markers. The scientists finished up with the supposition that the tamed genotypes of *P. vera* developed from 'Sarakhs' accessions.

Kolahi-Zonoozi et al. (2014) chipped away at the advancement of SSR markers all together for genetic diversity and structure analysis in pistachio. They revealed the advancement of 12 novel SSR markers segregated from a recurrent grouping enhanced genomic library of *P. vera*. They evaluated the genetic structure and a decent variety of 45 commercially significant Iranian cultivars utilizing 12 marker sets. The PIC changed from 0.19 to 0.56 with an average of 0.33. The mean estimation of observed heterozygosity (H_o) was 0.490, and the mean estimation of expected heterozygosity (H_e) was 0.345. They utilized a Neighbor-Net investigation that separated the 45 tested cultivars into two primary clusters. The outcomes from STRUCTURE software demonstrated the admixed hereditary qualities foundation of the tested samples. The authors inferred that these 12 recently created SSR markers may end up being helpful for future molecular breeding projects, and structure and diversity studies.

Sun et al. (2014) planned to discover sex markers in *P. chinensis* by RAPD and ISSR marker systems. Among 21 RAPD and 20 ISSR primers, two sex-explicit primers (S1 and S281) were distinguished, which had the option to intensify female-explicit fragments of 473 and 1242 bp, respectively. Be that as it may, just one fragment (FS281) was changed successfully into a SCAR marker. 636 bp fragment was enhanced by the SCAR primer combinations in all females while it was missing on the whole male samples at an annealing temperature of 64°C. The scientists expressed that their examination will offer a few clues to sex determination in *P. chinensis* plantations.

Kafkas et al. (2015) utilized restriction site-associated DNA (RAD) markers to explain the instrument of sex determination in pistachio and to recognize markers identified with sex in pistachio. F_1 population from a *P. vera* 'Siirt x Bağyolu' cross of eight females and eight males and their parents were utilized to sequence by Hi-Seq 2000 platform. Out of 37.7 Giga base (Gb) of sequences, 33,757 polymorphic SNP loci were distinguished between the parents. Thirty-eight of these sequences from 28 RAD reads were recognized as putative sex-related loci in pistachio. Among 13 tested markers, 8 of them could decide sex with 100% exactness in pistachio. As indicated by the SNaPshot analysis, five of the loci could not separate in F_1 offspring were disposed of. Further examination was finished utilizing the staying 8 SNP loci from 7 reads. Consequently, seven sets of SNP flanking primers were utilized in the high-resolution melt (HRM) investigation, which is another SNP genotyping strategy for a fast and savvy examination of hereditary variation. Among the seven tested SNP loci, four (SNP-PIS-133396, SNP-PIS-136404, SNP-PIS-167992, and SNP-PIS-174431) could recognize sex in every one of the 166 tested plants. While HRM investigation was not ready to decide sex in the remaining three SNP loci. Subsequently, four loci were recognized to be a legitimate apparatus for marker-assisted selection (MAS) in breeding programs. As a result of the female

heterogamety in all candidate SNP loci, they announced just because that pistachio has a ZZ/ZW sex-determination framework.

Zaloglu et al. (2015) created and portrayed SSR markers from pistachio and examined their transferability to eight *Pistacia* species. The specialists built a genomic library enhanced with CA, GA, AAC, and AAG repeats utilizing the genomic DNA of *P. vera* cv. 'Siirt' to create SSR markers for pistachio all together for deciding their transferability in eight pistachio species. An aggregate of 228 clones was sequenced by choosing 57 clones from every library, 68 were copies, though 94 contained repeats. The specialists structured 84 primer sets of which 59 created PCR products, and 47 were polymorphic among seven different pistachio cultivars. With an average of 3.6, the number of alleles ran from two to nine among 47 polymorphic loci. Among every one of the four built libraries, the GA-enhanced library was the best, though the AAC library was the most noticeably terrible as per the number of alleles, polymorphism, impeccable repeats, and instruction. Dinucleotide-enriched libraries apply a higher number and recurrence of SSRs than of the trinucleotide-enriched libraries. Testing of 59 SSR primer combines in eight *Pistacia* species indicated that 54 loci were transferable to in any event one of the tested *Pistacia* species. The most elevated number of transferable loci (51 loci) was accounted for in *P. atlantica*, trailed by *P. integerrima* (47 loci). The SSR loci had a higher transferability in the tri-nucleotide-enriched library (72.7%) than of the di-nucleotide-enriched library (54.7%). The authors presumed that the SSR markers created in their study are important apparatuses for hereditary investigations in pistachio and other related species later on.

Akdemir et al. (2016) surveyed the hereditary variety of long haul micropropagated develop pistachio created through direct shoot bud regeneration utilizing an apical bud and in vitro-determined leaves employing DNA-based molecular markers. RAPD, ISSR, and AFLP strategies were utilized in their examination. They acquired PIC estimations of 0.226, 0.220, and 0.241 produced by

RAPD, ISSR, and AFLP markers, separately. The outcomes indicated that the micropropagation of pistachio over various timeframes brought about “sensible polymorphism” among contributor plant and its 18 clones. Mantel’s test indicated a consistency polymorphism level between marker frameworks dependent on a similarity matrix. The authors reasoned this was the primary investigator in the event of a hereditary fluctuation in long haul micropropagated develop pistachio plantlets.

Guenni et al. (2016) considered genetic assorted variety examination of Tunisian pistachio utilizing SRAP markers. The analysts utilized 43 *P. vera* accessions that were screened by seven SRAP primer sets. They got an aggregate of 78 markers (95.12%) with an average PIC estimation of 0.850. The outcomes recommended that there was a solid genetic separation, which portrayed the local resources (G_{ST} = 0.307). An elevated level of gene flow (N_m = 1.127) among groups was clarified by the trading of plant materials among regions. Noteworthy contrasts inside groups were uncovered by examination of molecular variance that was 73.88% of the all-out genetic diversity inside the groups, while the remaining 26.12% happened among the groups. The authors utilized Bayesian clustering and principal component analysis that distinguished three pools, to be specific El Guettar, Pollenizers, and the remainder of the pistachios had a place with the Gabes, Kasserine, and Sfax regions. The Bayesian analysis uncovered that El Guettar and male genotypes were allocated with over 80% likelihood. The BayeScan strategy suggested that locus 59 (F13-R9) could be utilized in the advancement of sex-connected SCAR markers from SRAP since it was a regularly identified locus in examinations including the Pollenizers group. The authors expressed that this investigation was the main use of SRAP markers for the appraisal of genetic diversity in the Tunisian germplasm of *P. vera*.

Iranjo et al. (2016) examined hereditary decent variety and phylogenetic connections between and inside wild *Pistacia* species populations and suggestions for its preservation by utilizing the RAPD marker method. The specialists utilized

50 accessions from five wild *Pistacia* species included *P. vera*, *P. khinjuk*, *P. atlantica*, *P. mutica*, and *P. eurycarpa* that was broke down by RAPD markers. They watched a significant level of hereditary decent variety inside the tested accessions, as uncovered by utilizing UPGMA and molecular variance investigation. A sum of 146 (97.98%) PCR fragments was polymorphic out of 149 fragments. Genetic similarities ran from 0.3% to 0.86%, marker indices ran from 2.98% to 17.74%, and PIC ran from 0.80% to 0.99%. The investigators finished up the consequences of their investigation gave incredible molecular identification proof of all measured genotypes, which had indicated that there was an enormous amount of genetic diversity within the pistachio samples.

Motalebipour et al. (2016) overviewed the pistachio genome by next-generation sequencing (NGS) method that permitted them to create novel SSR markers and investigation of hereditary decent variety in *Pistacia* species. The scientists played out the study to investigate the genome structure utilizing the Illumina platform at roughly 40x inclusion depth in the *P. vera* cv. 'Siirt'. The K-mer investigation showed that pistachio has a genome that is around 600 Megabase (Mb) in size and is profoundly heterozygous. The assembly of 26.77 Gb Illumina data created 27,069 scaffolds at N_{50} = 3.4 kb with a sum of 513.5 Mb. A sum of 59,280 motifs was recognized with a recurrence of 8.67 kb. A sum of 206 SSR sets was utilized to describe 24 *P. vera* cultivars and 20 wild *Pistacia* genotypes having a place with *P. atlantica*, *P. integerrima*, *P. chinensis*, *P. terebinthus*, and *P. lentiscus* (four genotypes from each wild species). In general, 135 SSR loci intensified in every one of the 44 cultivars and genotypes in which 41 were polymorphic in six *Pistacia* species. The novel SSR loci created from cultivated pistachio were profoundly transferable to wild *Pistacia* species.

Topcu et al. (2016) created SSR markers for *P. vera* and related *Pistacia* species utilizing genomic libraries advanced with dinucleotide $(GA)_n$ and trinucleotide $(AAG)_n$ microsatellite motifs. For every library, an aggregate of 192

clones was sequenced, 135 primer sets were planned, and 110 created PCR products. While tested with 12 pistachio cultivars, 46 loci from the GA-improved library and 18 loci from the AAG-advanced library delivered polymorphic fragments. The polymorphic loci produced 264 alleles with an average of 4.13 alleles per locus. The PIC, H_o, and H_e were 0.51, 0.52, and 0.56, separately. While tested the transferability of 110 SSR primer sets in 10 wild *Pistacia* species, 100 were transferable to in any event one of the *Pistacia* species. The most elevated number of transferable loci was found in *P. eurycarpa*, while, the most minimal one was found in *P. texana* and *P. lentiscus*. The scientists presumed that their examination was the first of its sort to report the candidate SSR markers for *P. eurycarpa*, *P. palaestina*, and *P. texana*.

Jazi et al. (2017) researched the entire transcriptome sequence of *P. vera* uncovering novel bits of knowledge into saltiness related qualities and markers. The investigates utilized Illumina Hi-Seq 2000 platform that brought about 368,953,262 clean 100 bp paired-ends read (90 Gb) from 24 unique tissues of two pistachio cultivars. They made a few assemblies and surveyed the quality from a few points of view and found that utilizing the annotation-based matrix together with the length-based parameters permitted an improved appraisal of the transcriptome assembly quality. The created assembly by Trinity was received for functional annotation and subsequent examinations. The specialists clarified a sum of 29,119 contigs against every one of the five open databases, including NR (non-redundant), UniProt, TAIR10, KOG (eukaryotic orthologous group) and InterProScan. Among 279 KEGG (Kyoto Encyclopedia for Genes and Genomes), they further analyzed the pathways associated with the plant hormone biosynthesis and signaling just like those that added to the secondary metabolite biosynthesis because of their significance under saltiness stress. The specialists recognized 11,337 SSRs that were the most abundant dinucleotide repeats. Other than that, they distinguished 13,097 transcripts as candidate genes. They tentatively approved the declaration of these qualities through quantitative real-time PCR (qRT-PCR) that further affirmed the

exactness of the assembly. In light of this examination, the differentiating articulation pattern of NCED3 (9-cis-epoxycarotenoid dioxygenase-3) and SOS1 (son of sevenless homolog-1) genes were seen between salt-sensitive and salt-tolerant cultivars. The authors presumed that this present examination was the principal report in the whole transcriptome survey of *P. vera* that gave significant resources to functional and comparative genomic studies to find the saltiness tolerance-related markers and stress response mechanism for the pistachio breeding cultivars with more saltiness tolerance.

Kadivi et al. (2018) explored the hereditary decent variety of cultivated pistachio species by SSR markers. The specialists genotyped 42 pistachio cultivars utilizing 20 polymorphic nuclear SSR (nSSR) markers. The nSSR markers created 3-7 alleles (102 altogether) with an average of 5.10 per locus. The PIC esteem went from 0.36 to 0.86 with an average of 0.64. The H_o esteem extended from 0.21 to 0.79 with an average of 0.44, while the H_e esteem was 0.11 to 0.39 with an average of 0.22. They got the genetic similarities esteems from Dice's coefficient ran from 0.08 to 0.93. The built UPGMA dendrogram and Bayesian clustering isolated the examined cultivars into two significant groups that contained 21 and 21 cultivars, individually. The neighbor-joining strategy uncovered their phylogenetic connections. The specialists inferred that the outcomes from their current study demonstrated the presence of wide hereditary inconstancy inside the species and can be utilized for additional examination in the areas of germplasm protection and plant breeding.

Kadivi (2018) researched the hereditary decent variety of pistachio genotypes utilizing nSSR markers. The specialist saw that the transferability of the SSR markers was huge across genotypes, in this manner, flanking regions of the SSRs were conserved in the considered genotypes. They distinguished a significant level of polymorphism within the examined genotypes. An aggregate of 18 alleles was produced structure the seven SSR primer sets. Thirteen SSR primer sets were

polymorphic. The ranges of the polymorphic alleles were 1 (for Ptms9, Ptms40, Ptms41, and Ptms42 loci) to 5 (for Ptms7 locus) with an average of 2.57. The size of amplified alleles ran from 120 to 250 bp. Pair-wise hereditary comparability coefficients changed from 0.20 to 0.75. The UPGMA dendrogram isolated the tested genotypes into two significant distinct clusters. The specialist expressed that the outcomes acquired from the investigation might be utilized for the preservation, essence collection and future breeding programs of pistachio.

Choulak et al. (2019) utilized SSR markers to uncover the hereditary assorted variety and population structure in Tunisian pistachio. The scientists utilized three populations (El Guetar, Gafsa Sidi, and Bouzid) of Tunisian pistachio (42 accessions) utilizing a set of nine SSR markers. The examined genotypes uncovered 29 alleles with an average estimation of 2.9. The H_o and H_e for most of the locus showed that the frequencies of pistachio genotype got from the Hardy-Weinberg equilibrium. Notwithstanding their outcomes, they found that *P. atlantica* trees (Battoum) had explicit alleles intensified by two distinct primers. Factorial correspondence analysis (FCA) and neighbor-joining trees are organized autonomously from the plant's gender but organized based on the topographical source. The Bayesian investigation by STRUCTURE uncovered that all tested pistachio individuals could in all probability be relegated to two hereditary qualities pools to be specific, "South" and "Center". The authors commented that the uncovered SSR alleles were effectively used to separate various examined accessions. They additionally expressed that this investigation could be considered as a significant advance to guarantee the protection and the improvement of the National Database.

2.2. Studies on the Construction of Genetic Linkage Maps in *Pistacia*

Turkeli and Kafkas (2013) pioneered to build the first hereditary linkage map in pistachio utilizing an interspecific F_1 population having 92 individuals gotten from

a cross between *P. vera* cv. 'Siirt' and *P. atlantica* (monoecious genotype 'Pa-18'). 27 AFLP, 27 SRAP, and 16 ISSR primer sets were utilized by the authors to develop the 17 linkage groups in 'Siirt' and 19 linkage groups in 'Pa-18' genotype. A complete-length of 'Siirt' map was 1,237.3 centimorgan (cM) with 17 linkage groups and a mean marker density was determined 7.50 cM. 'Pa-18' map with 19 linkage groups covering 1,337.5 cM and an average marker density was 8.57 cM. Some of LGs (linkage groups) in parental maps were not very much immersed; in 'Siirt' map with four and in 'Pa-18' map with 5 minor LGs. The twofold pseudo-testcross procedure was utilized to build two separate hereditary linkage maps. An insufficient number of markers or individuals were most presumably cause the diminished recombination recurrence and accordingly made minor linkage groups, which cannot connect to a significant linkage. The authors recommended utilizing more markers with more individuals to determine the information into 15 linkage groups.

Khodaeiaminjan et al. (2017) played out an investigation that meant to create sex-connected SNP markers alongside expressed sequence tag-derived simple sequence repeats (EST-SSRs). They distinguished 9 novel sex-connected SNP markers by SNaPshot mini-sequencing investigation of 25 SNP loci from 17 RAD reads in 309 individuals. The F_1 population was gotten from crossing between *P. vera* cv. 'Siirt x Bağyolu'. The entirety of the 9 markers was heterozygous in female parents and homozygous in male parents that were in the long run upheld the ZW/ZZ sex-determination framework in pistachio. At long last, they developed a hereditary linkage map of the sex chromosome of pistachio. The consensus map was 65.2 cM long, while the female and male maps were 51.2 and 68.3 cM, individually. The gender locus was situated at 31.5 and 31.9 cM positions in the consensus as well as female maps, individually. The scientists expressed that the sex locus was close to the centromere of the sex-linked chromosome.

Khodaeiaminjan et al. (2018) built an SSR-based hereditary linkage map of pistachio. One-hundred and twenty-two F_1 offspring got from a cross between 'Siirt'

cultivar and ‘Bağyolu’ genotype was utilized in their examination. A sum of 1,027 SSR markers comprising of 625 novel loci and 402 previously created from different examinations were utilized to build the first reference map in pistachio. An aggregate of 384, 317, and 341 SSR markers were utilized to build the consensus, female and male maps separately spreading over 15 LGs for each map. The consensus map was 1,511.3 cM length with an average distance between markers was 3.9 cM and a 0.25 marker density. In ‘Siirt’ hereditary maps, the marker density was 0.22 marker per cM. ‘Siirt’ hereditary map had 15 linkage groups spreading over 1,427.0 cM lengths with 4.5 cM spanning between the markers. In ‘Bağyolu’ hereditary maps, marker density was 0.22 marker per cM. ‘Bağyolu’ hereditary map had 1,453.4 cM lengths with 4.2 cM spanning between the markers. The researchers additionally utilized common markers to develop the first SSR-based reference map in pistachio and 273 common markers were mapped alongside 15 linkage groups with an absolute length of 1,365.0 cM.

Motalebipour et al. (2018) made a consolidated hereditary linkage map utilizing all the SSR markers created to date in *Pistacia* species. The map was built utilizing 338 from 1,312 screened SSR markers on 15 linkage groups in 126 F_1 genotypes, which were obtained from the intercross of *P. vera* cv. ‘Siirt’ and monoecious *P. atlantica* cv. ‘Pa-18’. 306 SSR markers were mapped in ‘Siirt’ maternal hereditary map containing a length of 1,410.4 cM that spread over in 15 LGs with an average marker distance of 4.6 cM. The ‘Pa-18’ paternal hereditary map contained 285 SSR markers covered 1,362.5 cM that spanned in 15 LGs with an average markers distance of 4.8 cM. The scientists expressed that the built hereditary linkage maps right now be a significant resource for the future molecular and hereditary concerning study in pistachio.

2.3. Studies on Quantitative Trait Loci (QTL) in *Pistacia*

Motalebipour et al. (2018) contemplated the QTL investigation in pistachio for the first tıme. In their investigation, they utilized a hereditary linkage map built by SSR markers from the intercross between *P. vera* cv. 'Siirt' and monoecious *P. atlantica* cv. 'Pa-18' F_1 population. The phenotypic information was gathered in 2015 and 2016 developing seasons for four characteristics of leaf and shoot: leaf length (LL), leaf width (LW), leaf length/leaf width ratio (LWR), number of leaflet pairs (NLL), and young shoot color (YSC). They distinguished an aggregate of 17 QTLs in the parental maps. Four QTLs for LL and LW were situated on LG2 and LG4, while four QTLs for LWR on LG13 and LG14, two QTLs for NLL and two QTLs for YSC were on LG7 and LG9, separately, with identical positions in both parental maps.

3. MATERIAL AND METHODS

3.1. Plant Materials

An interspecific F_1 population derived from *P. vera* cv. 'Ohadi' and monoecious *P. atlantica* cv. 'Pa-18' ('Ohadi x Pa-18') genotype was used in this study for linkage mapping and QTL analysis. A total of 190 F_1 progenies were collected from Gaziantep Pistachio Research Institute in Turkey. The crosses between *P. vera* cv. 'Ohadi' and monoecious *P. atlantica* cv. 'Pa-18' ('Ohadi x Pa-18') was done in 2002 and the progenies were planted in 2004 (Kafkas et al. 2003) with a project supported by TÜBİTAK (Project No: TOVAG 100O113).

'Ohadi' has a typical morphological characteristic similar to *P. vera* with green leaves. 'Ohadi' is an Iranian cultivar that produces attractive nuts that are slightly smaller but tasty (Parfitt at al., 2012).

Monoecious *P. atlantica* cv. 'Pa-18' genotype is fully monoecious, which was reported by Kafkas et al. (2000), and used in the breeding program (Kafkas et al., 2003). 'Pa-18' genotype has a typical *P. atlantica* morphological characteristics with dark green leaves; the one-year shots are brownish and has a 3-4 leaflet pair. 'Pa-18' is shorter and narrower than 'Ohadi' cultivar.

3.2. Methods

3.2.1. Extraction of Genomic DNA

Genomic DNA from the gathered samples was extracted utilizing the CTAB (cetyltrimethylammonium bromide) technique (Doyle and Doyle, 1990) with minor alterations (Kafkas et al., 2006a). Young leaf tissues (approx. 1 g) of 190 offspring were utilized in this study. The leaves of each sample were ground utilizing TissueLyser II (Qiagen, the Netherlands) to a fine powder in liquid nitrogen and were then moved to 15 mL tube. At that point 6 mL CTAB buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 2% PVP, β-mercaptoethanol, and 0.1%

$Na_2S_2O_5$) was added to the squashed and ground sample in 15 mL tube followed by a 60 min incubation at 65°C in boiling water shower. The test samples were then mixed in with 6 mL chloroform-isoamyl alcohol (24:1) and centrifuged at 14,000 rpm for 15 min. The aqueous upper-layer was recouped and mixed with 2/3 volume of cold isopropanol to precipitate the DNA. The treated samples were kept at -20°C for 24 hours and afterward, isopropanol was poured off and the precipitated DNA was cleaned out with 10 mM ammonium acetic acid derivation in 76% ethanol. The supernatant was again expelled, and the pellet was dried at room temperature overnight. At that point, the dried DNA samples were diluted in 50 µL water (Thermo Scientific, HyClone – Molecular Biology Grade Water).

3.2.2. Quality Control and Quantification of Extracted DNA

The quality of the extracted genomic DNAs was controlled by 0.8% agarose gel. An aggregate of 10 µL mix (2 µL DNA and 18 µL loading dye) was stacked onto 0.8% agarose gel. The electrophoresis was set at 120 volts for 45 minutes. After electrophoresis, agarose gel was recolored with a fluorescent color (ethidium bromide), which ties to the nucleic acids. The stained gel was exposed to UV (ultraviolet) light source that allows DNA/dye to become visible. Based on the given bands of DNA to the gel image, the quality of DNA was determined. DNAs with a nice image and appropriate band were considered as the good ones whereas the DNAs with poor image and the inappropriate band were considered as bad ones and subjected to a second phase DNA extraction.

The measurement of concentration was performed using both the gel electrophoresis method and Qubit Fluorimeter (Invitrogen). In the gel electrophoresis, the concentration was estimated with a λ DNAs with known concentrations (25 ng, 50 ng, 100 ng, and 200 ng) standards. A total of 10 µL mix (2 µL DNA, and 18 µL loading dye) was loaded onto 0.8% agarose gel. The electrophoresis was set at 120 volts for 45 minutes. After electrophoresis, agarose

gel was stained with a fluorescent dye (ethidium bromide), which binds to the nucleic acid. The stained gel was exposed to a UV light source that allows DNA/dye to become visible. Comparing with λ DNAs in the gel image the extracted genomic DNA concentrations were determined.

Qubit Fluorimeter (Invitrogen) was used to determine the concentration of isolated genomic DNA as well as to validate the gel electrophoresis results. A 199 µL mix (198 µL buffer, and 1 µL dye) was prepared for each DNA sample. Then 198 µL of the mix was used along with 2 µL of DNA loaded into Qubit Fluorimeter to calculate the concentration in nanogram/milliliter (ng/mL). The isolated DNAs were then diluted to approximately 80 ng/µL and subjected to DArT-Seq™ analysis. DNA samples from a total of 190 genotypes that showed satisfactory quality and concentration were prepared for DArT-Seq™.

3.2.3. High-Throughput Genotyping by DArT-Seq™ Method

The isolated genomic DNA of 190 genotypes was sent to DArT PL (Diversity Array Technology, Pty Ltd), Canberra, Australia for genotyping. A high-throughput genotyping technique utilizing DArT-Seq™ innovation (Sansaloni et al., 2011) was utilized to genotype F_1 individuals. Two restriction enzymes *Pst*I and *Mse*I were chosen as enzyme combination to decrease genomic complexity. DNA samples were prepared in the digestion/ligation reactions as per Kilian et al. (2012), notwithstanding, the substitution of a single *Pst*I-compatible adaptor with two unique adaptors related to two distinctive restriction enzyme (RE) overhangs. The *Pst*I-compatible adaptor was intended to incorporate Illumina flowcell connection sequence, sequencing primers, and "staggered", differing length barcode region, like the sequence announced by Elshire et al. (2011). The reverse adaptor contained the flowcell connection region and *Mse*I-compatible overhang sequence. Just "mixed fragments" (*Pst*I–*Hpa*II) were adequately enhanced in 30 rounds of PCR utilizing the accompanying reaction conditions: 1 min at 94°C for beginning denaturation; 30

cycles each comprising of 20 s at 94°C for denaturation, 30 s at 58°C for annealing and 45 s at 72°C for expansion, lastly a 7 min augmentation step at 72°C. After PCR, equimolar measures of amplified items from each sample of the 96-well microtiter plate were bulked and applied to c-Bot (Illumina) connect PCR followed by sequencing on the Illumina Hi-Seq 2500 platform. The single-read sequencing was run for 77 cycles.

3.2.4. SNP Calling

Sequences produced from every path of Illumina Hi-Seq 2500 were handled utilizing restrictive DArT explanatory pipelines. In the primary pipeline, the FASTQ files were first handled to filter low-quality sequences, applying progressively stringent determination criteria to the barcode region contrasted with the remainder of the sequence. Subsequently, the assignments of the sequence to explicit samples conveyed in the "barcode split" step are progressively steady. Roughly 2,500,000 (±7%) arrangements per barcode/sample were utilized in marker call. At last, indistinguishable sequences were collapsed into FASTQCOL files. These files were utilized in the second pipeline for DArT PL's restrictive SNP calling algorithms (DArTsoft14) to yield candidate SNP markers that are polymorphic within the arrangement of test samples. Every unique sequence from the set of FASTQCOL files are distinguished and clustered by sequence similarity at a distance threshold of 3 base variations. The sequence clusters are then parsed into SNP markers using a scope of metadata parameters got from the quantity and distribution of each sequence over all samples in the examination. The candidate markers yield by DArTsoft14 are additionally filtered based on the reproducibility values, average count for each sequence or row sum (sequencing depth), the equalization of average counts for every SNP allele, and the call rate (extent of samples for which the marker is scored).

3.2.5. Construction of Genetic Linkage Maps and QTL Analysis

SNP markers acquired from DArT-Seq™ examination were screened to decide segregation patterns utilizing female and male parents. Parental markers (lmxll, and nnxnp) were utilized in linkage map development. Linkage maps were developed by utilizing the twofold pseudo-testcross mapping procedure (Grattapaglia and Sederoff, 1994) for each parent autonomously. SNP markers from DArT PL (Diversity Array Technology, Pty Ltd in Australia) obtained under the pistachio genome project by Kafkas et al. (2020). To perform QTL analysis, linkage analysis was again performed using JoinMap v4.1 (Van Ooijen, 2013) software. The mapped markers by Kafkas et al. (2020) were used to reconstruct the parental linkage maps. The chi-square (χ^2) test for every locus was applied to test for the goodness of fit the segregating markers of Mendelian ratios hypothesis (Van Ooijen, 2013). The LOD (log of odds) scores dependent on G2 statistics determined to utilize JoinMap for the recombination frequency to decide the linkage groups. In the mapping procedure utilizing JoinMap, a couple of markers were disposed of from the last maps because of the trouble to decide their linkage stages. Markers were likewise expelled when their segregation pattern clashed with the isolation sample of neighboring markers.

To decide the linkage edge of markers, a LOD score of 5.0 was set at first for all maps in a similar linkage group by standard JoinMap parameter settings. All together for expressing recombination frequencies as a linkage distance in cM of maps, Kosambi's mapping function (Kosambi, 1944) and regression mapping algorithm were utilized for map development. The visualization of the maps was finished utilizing MapChart v2.2 for the Windows platform (Voorrips, 2002).

3.2.6. Phenotypic Characterization and QTL Analysis

A total of 18 phenotypic characteristics were observed in the F_1 population. Measurement or observations on leaf length (LL), leaf width (LW), number of

leaflets (NL), terminal leaflet length (TLL), terminal leaflet width (TLW), terminal leaflet shape (TLS), terminal leaflet size (TLSZ), terminal leaflet apex (TLA), terminal leaflet base (TLB), tree vigor (TV), one-year-old shoot color (OYOSC), date of first bud burst (DFBB), inflorescence abundance (IA), first bloom date (FBD), peak bloom date (PBD), last bloom date (LBD), date of vegetative bud break (DVBB), and inflorescence rachis length (IRL) were performed in the F_1 progenies in 2018 and 2019 growing seasons. The LL, LW, TLL, TLW, and IRL of ten representative mature leaves from each progeny were measured by ruler and the means were calculated to represent the trait. Ten representative leaflets for each tree were counted for NL. Most of the phenotypic data were collected and categorized for the analysis according to Descriptors for Pistachio (IPGRI, 1997). TLS was determined to observe 10 representative leaves based on pistachio descriptors into six categories: broad lanceolate [1], elliptic [2], ovate [3], round ovate [4], roundish [5], and others [99]. TLSZ was observed from 10 representative leaves into three categories: similar to basal leaflets [1], larger than basal leaflets [2], and smaller than basal leaflets [3]. TLA was determined into six categories: accuminate [1], mucronate [2], mucronulate [3], obtuse [4], retuse [5], and others [99]. TLB was observed from 10 representative leaves into five different categories: attenuate [1], obtuse [2], truncate [3], oblique [4], and others [5]. The TV was observed into three categories: low [3], intermediate [5], and high [7]. The OYOSC was determined by observing the F_1 progenies into three categories: light brown [1], brown [2], and dark brown [3]. IA was determined into three categories: sparse [3], intermediate [5], and dense [7]. DFBB, FBD, PBD, LBD, and DVBB were calculated by subtracting the first day of the observed year from the original observed day. A statistical analysis of phenotypic data was performed using Microsoft Excel 2016 and SPSS v25. The correlation between measurements and distribution frequency of all 18 observed traits was calculated during the 2018 and 2019 growing seasons.

MapQTL v5.0 (Van Ooijen, 2008) was utilized to examine the QTLs. Utilizing an interval mapping approach; the recognition of QTLs was finished by hereditary linkage maps for each parent. At first, the nearest marker to the LOD pinnacle of each significant QTL in the interval map (IM) was utilized as a cofactor. At that point, multiple QTL mapping (MQM) was performed. Putative QTLs with LOD scores more prominent than 2.0 in datasets were accounted for. The LOD threshold at the genome-wide level was controlled by running the 1000 permutation test. Besides, the Kruskal-Wallis test (KW test) was performed to check the recognized QTLs.

4. RESULTS AND DISCUSSION

The high-density genetic linkage maps of the F_1 population derived from *P. vera* cv. 'Ohadi' x *P. atlantica* cv. 'Pa-18' has been constructed in this study based on SNP markers obtained from DarT-Seq™. A total of 190 F_1 populations were used in this study.

4.1. The Genetic Linkage Maps in Pistachio Using SNP Markers

Genetic linkage maps in pistachio were constructed using SNP markers in this study. Among 2,112 SNP markers obtained from DArT-Seq™, 1,407 SNP markers were mapped in 'Ohadi'. A total of 2,518 SNP markers were mapped in the LGs of 'Pa-18' among 3,062 SNP markers obtained from DArT-Seq™. Fifteen LGs were constructed from 'Ohadi x Pa-18' haploid chromosomes. The segregation type and pattern of 'Ohadi x Pa-18' genetic maps constructed by SNP markers have been shown in Table 4.1. The illustrations of LGs are shown below from Figure 4.1 to Figure 4.15.

Table 4.1. The segregation type, segregation pattern, and the number of the locus for 'Ohadi' and 'Pa-18' genetic maps.

Parent	Segregation type	Segregation pattern	Number of mapped loci
'Ohadi' female	<lmxll>	lm, ll (1:1)	1,407
'Pa-18' male	<nnxnp>	nn, np (1:1)	2,518

4.1.1. LG1

The LG1 of 'Ohadi x Pa-18' has been shown in Figure 4.1. The LG1 of 'Ohadi' was constructed by 208 SNP markers with a total length of 104.7 cM. The average distance between markers was 0.5 cM and the average marker density was 1.99 markers per cM. The number of distorted markers was 24 (11.5%). The largest

gap was 4.5 cM between the markers 11982321 and 7266384 (Figure 4.1 and Supplementary File 1).

Figure 4.1. The LG1 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG1 of 'Pa-18' was constructed by 303 SNP markers with a total map length of 127.8 cM. The average distance between markers was 0.4 cM and the average marker density was 2.37 markers per cM. The number of distorted markers was 57 (18.8%). The largest gap was 7.4 cM between the markers 7287414 and 7284139 (Figure 4.1 and Supplementary File 2).

4.1.2. LG2

The LG2 of 'Ohadi x Pa-18' has been shown in Figure 4.2. The LG2 of 'Ohadi' was constructed by 49 SNP markers with a total map length of 57.7 cM. The average distance between markers was 1.2 cM and the average marker density was 0.85 markers per cM. None of the markers were distorted. The largest gap was 6.2 cM between the markers 7289514 and 11985944 (Figure 4.2 and Supplementary File 3).

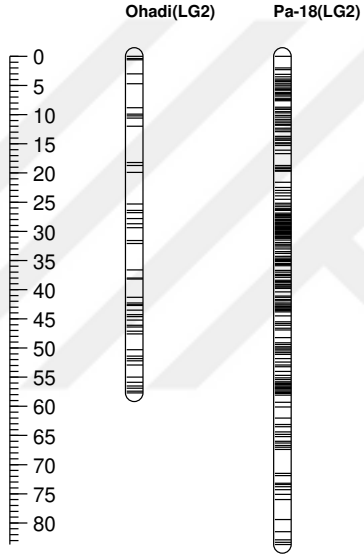

Figure 4.2. The LG2 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG2 of 'Pa-18' was constructed by 253 SNP markers with a total map length of 83.7 cM. The average distance between markers was 0.3 cM and the average marker density was 3.02 markers per cM. The number of distorted markers was 125 (49.4%). The largest gap was 4.2 cM between the markers 7289389 and 7265924 (Figure 4.2 and Supplementary File 4).

4.1.3. LG3

The LG3 of 'Ohadi x Pa-18' has been shown in Figure 4.3. The LG3 of 'Ohadi' was constructed by 61 SNP markers with a total map length of 67.5 cM. The average distance between markers was 1.1 cM and the average marker density was 0.90 markers per cM. The number of distorted markers was only one (1.4%). The largest gap was 4.9 cM between the markers 7272679 and 7286314 (Figure 4.3 and Supplementary File 5).

Figure 4.3. The LG3 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG3 of 'Pa-18' was constructed by 194 SNP markers with a total map length of 86.3 cM. The average distance between markers was 0.4 cM and the average marker density was 2.25 markers per cM. The number of distorted markers was 33 (17.0%). The largest gap was 4.5 cM between the markers 7281654 and 7263213 (Figure 4.3 and Supplementary File 6).

4.1.4. LG4

The LG4 of 'Ohadi x Pa-18' has been shown in Figure 4.4. The LG4 of 'Ohadi' was constructed by 125 SNP markers with a total map length of 109.0 cM. The average distance between markers was 0.9 cM and the average marker density was 1.15 markers per cM. The number of distorted markers was 41 (32.8%). The largest gap was 24.3 cM between the markers 11981661 and 7274011 (Figure 4.4 and Supplementary File 7).

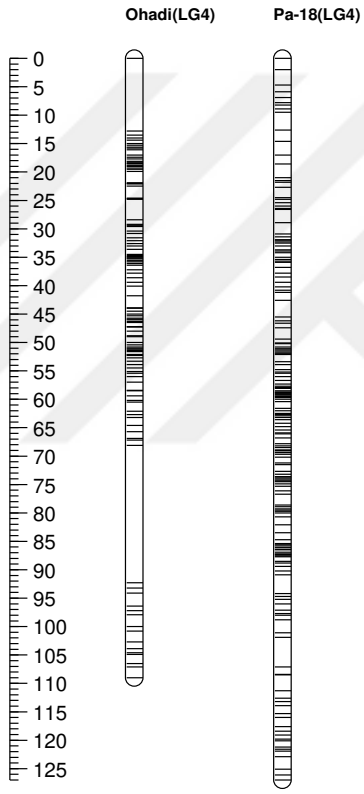

Figure 4.4. The LG4 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG4 of 'Pa-18' was constructed by 186 SNP markers with a total map length of 127.0 cM. The average distance between markers was 0.7 cM and the

average marker density was 1.46 markers per cM. The number of distorted markers was 27 (14.5%). The largest gap was 5.2 cM between the markers 7283332 and 7284118 (Figure 4.4 and Supplementary File 8).

4.1.5. LG5

The LG5 of 'Ohadi x Pa-18' has been shown in Figure 4.5. The LG5 of 'Ohadi' was constructed by 131 SNP markers with a total map length of 73.1 cM. The average distance between markers was 0.6 cM and the average marker density was 1.79 markers per cM. The number of distorted markers was 3 (2.3%). The largest gap was 4.1 cM between the markers 7283405 and 11984848 (Figure 4.5 and Supplementary File 9).

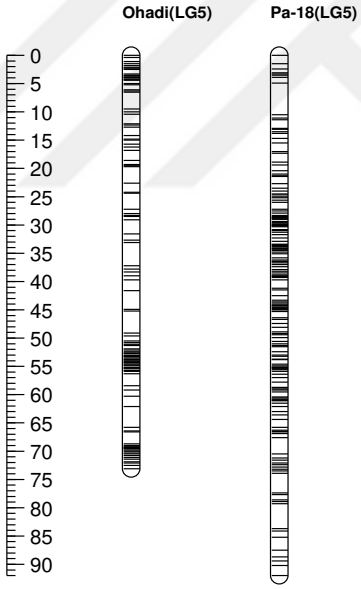

Figure 4.5. The LG5 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG5 of 'Pa-18' was constructed by 190 SNP markers with a total map length of 92.0 cM. The average distance between markers was 0.5 cM and the average marker density was 2.06 markers per cM. The number of distorted markers was 64 (33.7%). The largest gap was 5.5 cM between the markers 7279779 and 7283134 (Figure 4.5 and Supplementary File 10).

4.1.6. LG6

The LG6 of 'Ohadi x Pa-18' has been shown in Figure 4.6. The LG6 of 'Ohadi' was constructed by 87 SNP markers with a total map length of 71.0 cM. The average distance between markers was 0.8 cM and the average marker density was 1.22 markers per cM. The number of distorted markers was 5 (5.7%). The largest gap was 4.6 cM between the markers 7275256 and 11988729 (Figure 4.6 and Supplementary File 11).

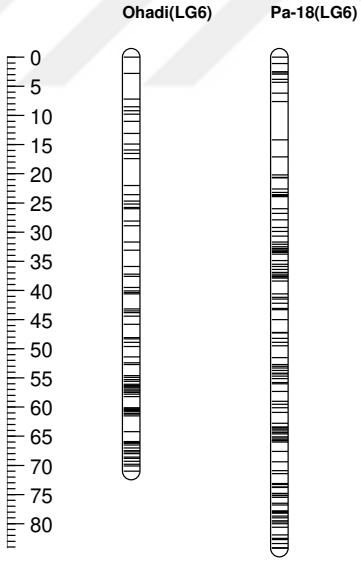

Figure 4.6. The LG6 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG6 of 'Pa-18' was constructed by 133 SNP markers with a total map length of 84.2 cM. The average distance between markers was 0.6 cM and the average marker density was 1.58 markers per cM. The number of distorted markers was 76 (57.1%). The largest gap was 6.6 cM between the markers 7285135 and 7282594 (Figure 4.6 and Supplementary File 12).

4.1.7. LG7

The LG7 of 'Ohadi x Pa-18' has been shown in Figure 4.7. The LG7 of 'Ohadi' was constructed by 76 SNP markers with a total map length of 84.7 cM. The average distance between markers was 1.1 cM and the average marker density was 0.90 markers per cM. The number of distorted markers was 5 (6.6%). The largest gap was 9.9 cM between the markers 7252248 and 7275618 (Figure 4.7 and Supplementary File 13).

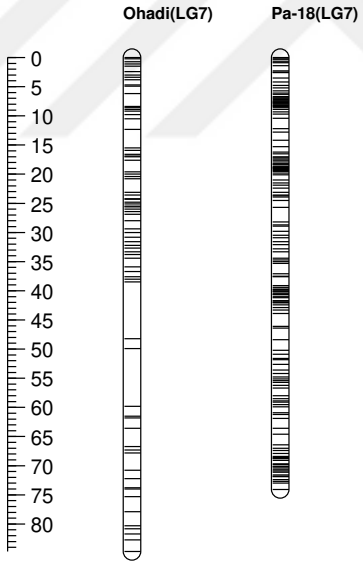

Figure 4.7. The LG7 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG7 of 'Pa-18' was constructed by 180 SNP markers with a total map length of 74.1 cM. The average distance between markers was 0.4 cM and the average marker density was 2.43 markers per cM. There were no distorted markers in LG7. The largest gap in the 'Pa-18' LG7 map was 2.5 cM between the markers 7264797 and 7282937 (Figure 4.7 and Supplementary File 14).

4.1.8. LG8

The LG8 of 'Ohadi x Pa-18' has been shown in Figure 4.8. The LG8 of 'Ohadi' was constructed by 136 SNP markers with a total map length of 75.4 cM. The average distance between markers was 0.6 cM and the average marker density was 1.80 markers per cM. The number of distorted markers was 61 (44.9%). The largest gap was 3.6 cM between the markers 7282102 and 11982964 (Figure 4.8 and Supplementary File 15).

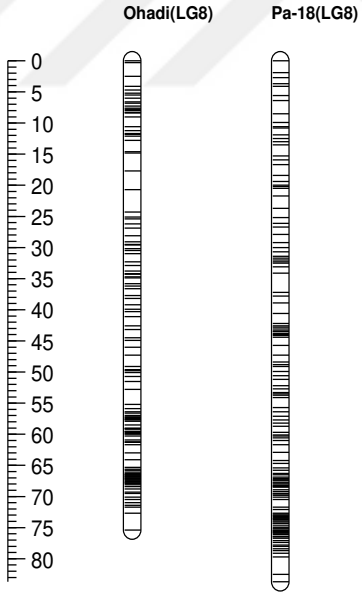

Figure 4.8. The LG8 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG8 of 'Pa-18' was constructed by 160 SNP markers with a total map length of 83.7 cM. The average distance between markers was 0.5 cM and the average marker density was 1.91 markers per cM. The number of distorted markers was 8 (5.0%). The largest gap was 3.1 cM between the markers 7282584 and 7279975 (Figure 4.8 and Supplementary File 16).

4.1.9. LG9

The LG9 of 'Ohadi x Pa-18' has been shown in Figure 4.9. The LG9 of 'Ohadi' was constructed by 62 SNP markers with a total map length of 73.0 cM. The average distance between markers was 1.2 cM and the average marker density was 0.85 markers per cM. There was no distorted marker in this group. The largest gap in 'Ohadi' LG9 map was 6.2 cM between the markers 7282316 and 11984226 (Figure 4.9 and Supplementary File 17).

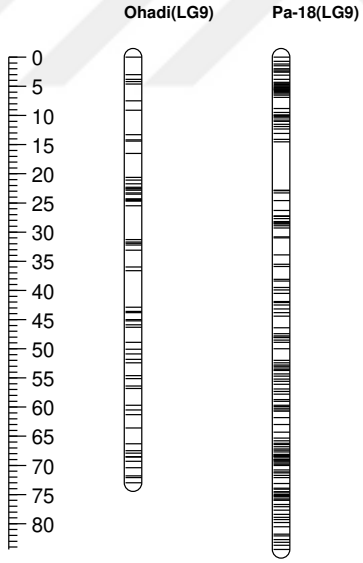

Figure 4.9. The LG9 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is illustrated on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG9 of 'Pa-18' was constructed by 163 SNP markers with a total map length of 84.4 cM. The average distance between markers was 0.5 cM and the average marker density was 1.93 markers per cM. The number of distorted markers was 3 (1.8%). The largest gap in the 'Pa-18' LG9 map was 8.2 cM between the markers 7265783 and 7269352 (Figure 4.9 and Supplementary File 18).

4.1.10. LG10

The LG10 of 'Ohadi x Pa-18' has been shown in Figure 4.10. The LG10 of 'Ohadi' was constructed by 113 SNP markers with a total map length of 82.2 cM. The average distance between markers was 0.7 cM and the average marker density was 1.37 markers per cM. The number of distorted markers was 56 (49.6%). The largest gap was 11.6 cM between the markers 11984896 and 7266447 (Figure 4.10 and Supplementary File 19).

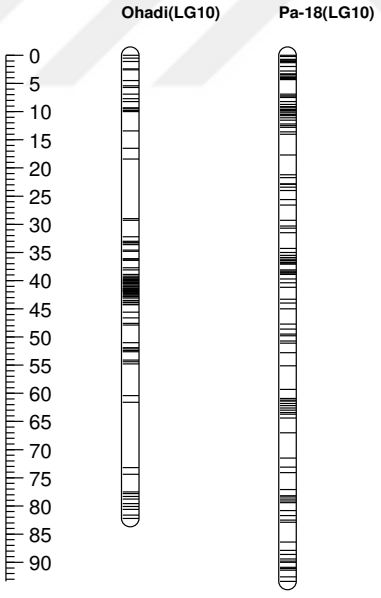

Figure 4.10. The LG10 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is shown on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG10 of 'Pa-18' was constructed by 148 SNP markers with a total map length of 93.4 cM. The average distance between markers was 0.6 cM and the average marker density was 1.58 markers per cM. The number of distorted markers was 70 (47.3%). The largest gap was 4.5 cM between the markers 11989096 and 7280156 (Figure 4.10 and Supplementary File 20).

4.1.11. LG11

The LG11 of 'Ohadi x Pa-18' has been shown in Figure 4.11. The LG11 of 'Ohadi' was constructed by 62 SNP markers with a total map length of 55.1 cM. The average distance between markers was 0.9 cM and the average marker density was 1.13 markers per cM. There were no distorted markers in LG11. The largest gap was 11.9 cM between the markers 7266438 and 7279080 (Figure 4.11 and Supplementary File 21).

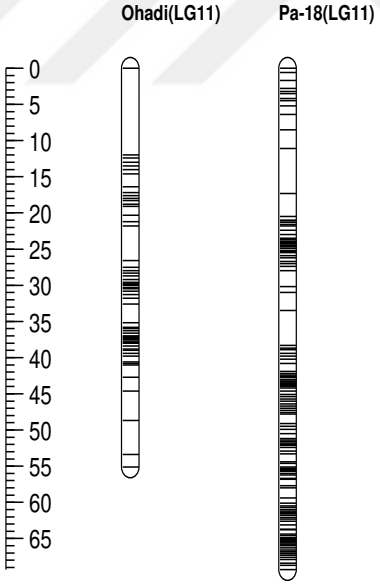

Figure 4.11. The LG11 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is shown on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG11 of 'Pa-18' was constructed by 147 SNP markers with a total map length of 69.3 cM. The average distance between markers was 0.5 cM and the average marker density was 2.12 markers per cM. The number of distorted markers was 3 (2.0%). The largest gap was 6.2 cM between the markers 11992100 and 7280991 (Figure 4.11 and Supplementary File 22).

4.1.12. LG12

The LG12 of 'Ohadi x Pa-18' has been shown in Figure 4.12. The LG12 of 'Ohadi' was constructed by 85 SNP markers with a total map length of 82.2 cM. The average distance between markers was 1.0 cM and the average marker density was 1.03 markers per cM. The number of distorted markers was 7 (8.2%). The largest gap was 6.3 cM between the markers 11985468 and 7281839 (Figure 4.12 and Supplementary File 23).

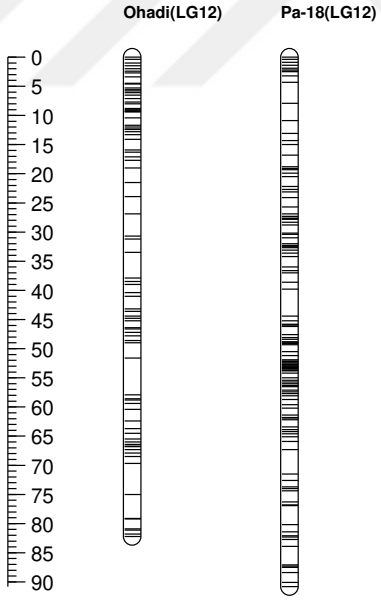

Figure 4.12. The LG12 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is shown on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG12 of 'Pa-18' was constructed by 132 SNP markers with a total map length of 90.8 cM. The average distance between markers was 0.7 cM and the average marker density was 1.45 markers per cM. The number of distorted markers was 77 (58.3%). The largest gap was 4.6 cM between the markers 7278691 and 11988408 (Figure 4.12 and Supplementary File 24).

4.1.13. LG13

The LG13 of 'Ohadi x Pa-18' has been shown in Figure 4.13. The LG13 of 'Ohadi' was constructed by 79 SNP markers with a total map length of 61.5 cM. The average distance between markers was 0.8 cM and the average marker density was 1.29 markers per cM. The number of distorted markers was 4 (5.1%). The largest gap was 8.7 cM between the markers 7284917 and 7278109 (Figure 4.13 and Supplementary File 25).

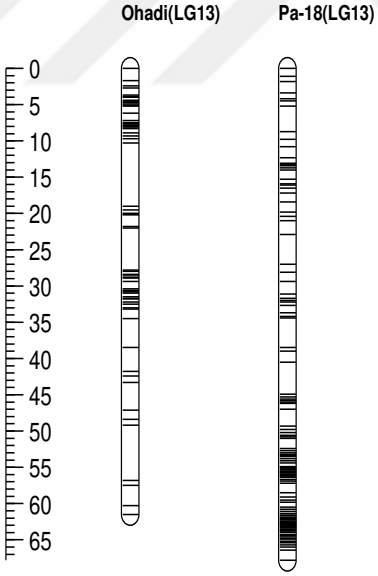

Figure 4.13. The LG13 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is shown on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG13 of 'Pa-18' was constructed by 138 SNP markers with a total map length of 67.8 cM. The average distance between markers was 0.5 cM and the average marker density was 2.04 markers per cM. The number of distorted markers was 13 (9.4%). The largest gap was 4.4 cM between the markers 7288267 and 7272318 (Figure 4.13 and Supplementary File 26).

4.1.14. LG14

The LG14 of 'Ohadi x Pa-18' has been shown in Figure 4.14. The LG14 of 'Ohadi' was constructed by 44 SNP markers with a total map length of 57.1 cM. The average distance between markers was 1.3 cM and the average marker density was 0.77 markers per cM. The number of distorted markers was 13 (29.5%). The largest gap was 9.1 cM between the markers 7287111 and 7279326 (Figure 4.14 and Supplementary File 27).

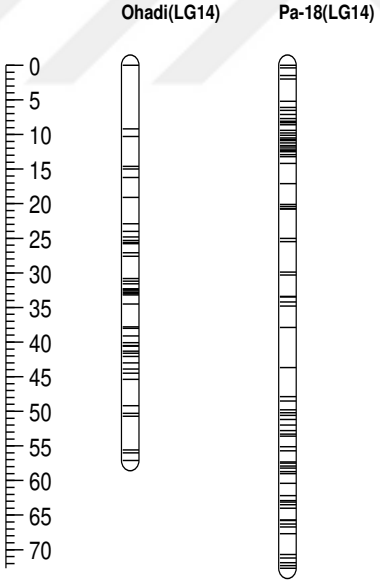

Figure 4.14. The LG14 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is shown on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG14 of 'Pa-18' was constructed by 77 SNP markers with a total map length of 72.7 cM. The average distance between markers was 0.9 cM and the average marker density was 1.06 markers per cM. The number of distorted markers was 5 (6.5%). The largest gap in the 'Pa-18' LG14 map was 5.8 cM between the markers 7264510 and 7277625 (Figure 4.14 and Supplementary File 28).

4.1.15. LG15

The LG15 of 'Ohadi x Pa-18' has been shown in Figure 4.15. The LG15 of 'Ohadi' was constructed by 89 SNP markers with a total map length of 67.9 cM. The average distance between markers was 0.8 cM and the average marker density was 1.31 markers per cM. The number of distorted markers was 34 (38.0%). The largest gap was 3.4 cM between the markers 11992311 and 7264694 (Figure 4.15 and Supplementary File 29).

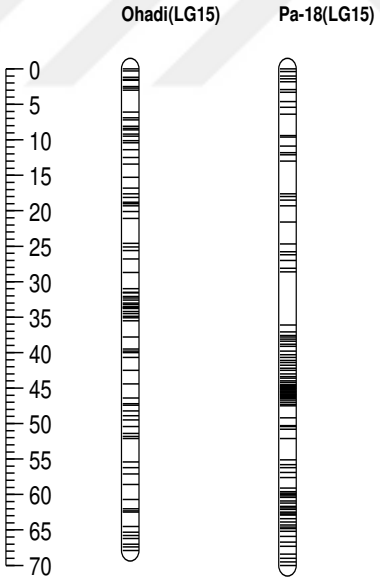

Figure 4.15. The LG15 of 'Ohadi x Pa-18' F_1 population. 'Ohadi' map is illustrated on the left side whereas the 'Pa-18' map is shown on the right side. The left-sided numbers in the scale indicate the distances of markers as cM.

The LG15 of 'Pa-18' was constructed by 114 SNP markers with a total map length of 70.0 cM. The average distance between markers was 0.6 cM and the average marker density was 1.63 markers per cM. The number of distorted markers was 24 (21.1%). The largest gap was 7.5 cM between the markers 7288598 and 7285306 (Figure 4.15 and Supplementary File 30).

4.2. Summary of 'Ohadi x Pa-18' Genetic Linkage Maps Constructed by SNP Markers

The separate 15 LGs for each parent were constructed using SNP markers in 'Ohadi x Pa-18' F_1 population. The length of LG, the number of markers, the distance between markers, marker density, and distorted markers for each LG were presented in Table 4.2.

In the 'Ohadi' female map, a total of 1,407 SNP markers were mapped in a total length of 1,122.2 cM with an average of 74.8 cM. The LG4 (109.0 cM) and LG1 (104.7 cM) were the longest LG, while LG11 (55.1 cM) was the shortest one. The average number of markers in each group was 93.8, where LG1 had the maximum (208), and LG14 had the minimum (44) number of mapped markers. With an average of 0.9 cM, the lowest distance between markers was in LG1 (0.5 cM), while the highest distance between markers was in LG14 (1.3 cM). The highest marker density was in LG1 (1.99 per cM) and LG8 (1.80 per cM), while the lowest marker density was in LG14 (0.77 per cM), LG2 (0.85 per cM), and LG9 (0.85 per cM) with an average of 1.22 per cM. The markers in LG10 and LG8 had the highest distortion rates with 49.6% and 44.9%, respectively, while no markers were distorted in LG2, LG9, and LG11 (Table 4.2). The average distortion rate was 15.7%.

In the 'Pa-18' male map, a total of 2,518 SNP markers were mapped in a total length of 1,307.2 cM with an average of 87.1 cM. The LG1 (127.8 cM) and LG4 (127.0 cM) were the longest LGs, while LG13 (67.8 cM) and LG11 (69.3 cM) were the shortest ones. The average number of markers in each linkage group was

167.9, where LG1 (303) and LG2 (253) had the highest, and LG14 (77) had the lowest number of markers. The lowest distance between markers was in LG2 (0.3 cM), while the highest distance between markers was in LG14 (0.9 cM) with an average of 0.5 cM. The highest marker density was in LG2 (3.02 per cM) while the lowest marker density was in LG14 (1.06 per cM) with an average of 1.93 per cM. The markers of LG12 and LG6 had the highest distortion rate of 58.3% and 57.1%, respectively while LG7 had no distorted markers (Table 4.2).

Table 4.2. The length of LG, number of markers, distance between markers, marker density, and rate of distorted markers in 'Ohadi' and 'Pa-18' genetic maps constructed by SNP markers.

'Ohadi'						'Pa-18'					
LG	LG length (cM)	Number of markers	Distance between markers (cM)	Marker density	Rate of distorted markers (%)	LG	LG length (cM)	Number of markers	Distance between markers (cM)	Marker density	Rate of distorted markers (%)
1	104.7	208	0.5	1.99	11.5	1	127.8	303	0.4	2.37	18.8
2	57.7	49	1.2	0.85	0.0	2	83.7	253	0.3	3.02	49.4
3	67.5	61	1.1	0.90	1.6	3	86.3	194	0.4	2.25	17.0
4	109.0	125	0.9	1.15	32.8	4	127.0	186	0.7	1.46	14.5
5	73.1	131	0.6	1.79	2.3	5	92.0	190	0.5	2.06	33.7
6	71.0	87	0.8	1.22	5.7	6	84.2	133	0.6	1.58	57.1
7	84.7	76	1.1	0.90	6.6	7	74.1	180	0.4	2.43	0.0
8	75.4	136	0.6	1.80	44.9	8	83.7	160	0.5	1.91	5.0
9	73.0	62	1.2	0.85	0.0	9	84.4	163	0.5	1.93	1.8
10	82.2	113	0.7	1.37	49.6	10	93.4	148	0.6	1.58	47.3
11	55.1	62	0.9	1.13	0.0	11	69.3	147	0.5	2.12	2.0
12	82.2	85	1.0	1.03	8.2	12	90.8	132	0.7	1.45	58.3
13	61.5	79	0.8	1.29	5.1	13	67.8	138	0.5	2.04	9.4
14	57.1	44	1.3	0.77	29.5	14	72.7	77	0.9	1.06	6.5
15	67.9	89	0.8	1.31	38.0	15	70.0	114	0.6	1.63	21.1
Total	1,122.2	1,407				Total	1,307.2	2,518			
Average	74.8	93.8	0.9	1.22	15.7	Average	87.1	167.9	0.5	1.93	22.8

Kafkas et al. (2015) studied the SNP marker system for the first time to develop sex-linked markers in pistachio. The authors used RAD markers and identified 8 sex-linked SNP markers, and reported for the first-time pistachio having a ZW sex-determination system. Khodaeiaminjan et al. (2017) mapped SNP markers together with SSRs using an intra-specific mapping population (*P. vera* cv. 'Siirt x Bağyolu'). The authors defined sex chromosomes of pistachio with a total length of 65.1 cM. The sex locus and sex-linked SNPs were positioned in the center of the map at a distance of 31.8 and 31.9 cM respectively.

Three published literature has been found to date regarding the genetic linkage mapping in pistachio using AFLP, ISSR, SRAP, and SSR marker systems. Turkeli and Kafkas (2013) constructed the first genetic linkage maps from a cross between *P. vera* cv. 'Siirt' and monoecious *P. atlantica* cv. 'Pa-18' ('Siirt x Pa-18') using 107 AFLP, 9 ISSR, and 49 SRAP markers in 'Siirt' maps, and 99 AFLP, 10 ISSR, and 47 SRAP markers in 'Pa-18' maps respectively. The total number of markers was 165 and 156 for 'Siirt' and 'Pa-18' respectively. The average number of markers was 9.70 and 8.21 per LG for 'Siirt' and 'Pa-18' respectively. The average marker density was 7.50 and 8.57 per LG for 'Siirt' and 'Pa-18' respectively. The number of linkage groups was 17 for 'Siirt' and 19 for 'Pa-18'. The total length of the maps was 1,237.3 cM and 1,337.5 cM for 'Siirt' and 'Pa-18' respectively. The average length of linkages was 72.7 cM for 'Siirt' and 70.3 cM for 'Pa-18'. The largest gap on maps was 41.4 cM for 'Siirt' and 40.4 cM for 'Pa-18' maps. The total number of gaps (>20 cM) was 12 and 21 for 'Siirt' and 'Pa-18' maps respectively.

Khodaeiaminjan et al. (2018) constructed the consensus and parental genetic linkage maps of pistachio using pseudo-testcross mapping strategy that spanned to 15 linkage groups each. The researchers used the F_1 population obtained from a cross between *P. vera* cv. 'Siirt' and cv. 'Bağyolu' ('Siirt x Bağyolu'). The resulting consensus map was 1,511.3 cM in length with an average of 100.8 cM per LG. The longest LG was 131.0 cM long while the shortest LG was 61.4 cM long. The number

of mapped SSR markers varied from 16 to 40 with an average of 25.6. In the consensus map, the average distance between SSR markers was 3.9 cM. The average marker density was 0.25 per cM. There were four gaps in the consensus maps larger than 20 cM. In the case of the 'Siirt' maternal maps, 15 LGs were constructed by 317 SSR markers with an average of 21.1 markers in each LG varied from 11 to 33 markers. The 'Siirt' map had a total length of 1,427.0 cM with a mean of 95.1 cM ranged from 37.3 cM and 130.7 cM. The average distance between SSR markers in the 'Siirt' map was 4.5 cM that ranged from 2.9 cM to 6.2 cM. An average density of 0.22 markers per cM was counted for maternal maps. There were six gaps larger than 20 cM on five LGs in maternal maps. On the other hand, 'Bağyolu' paternal linkage maps were constructed by 341 SSR markers with an average of 22.7 markers per LG. The total length was 1,453.4 cM with an average of 96.7 cM. The lengths of the LGs varied from 58.2 cM to 132.7 cM. The average distances between markers of the 'Bağyolu' map varied from 3.1 cM to 6.1 cM with an average of 4.2 cM. There were four gaps larger than 20 cM on three LGs in paternal maps.

Motalebipour et al. (2018) constructed parental genetic linkage maps of 15 LGs from each parent in pistachio using an interspecific F_1 population derived from *P. vera* cv. 'Siirt' and *P. atlantica* cv. 'Pa-18' ('Siirt x Pa-18'). The authors screened the segregation of 1,312 SSR primer pairs and 388 SSRs were successfully mapped. Of the 1,312 screened primer pairs, 1,263 primer pairs were developed from *P. vera*, whereas, rest of the primer pairs were developed from wild *Pistacia* species. Overall, 203 of the 388 mapped SSRs were heterozygous in both parents. In the case of the 'Siirt' female maps, 306 SSRs were successfully mapped to the 15 LGs. In total, 203 common markers were mapped giving a total map length of 1,410.4 cM. With an average length of 94.0 cM, the LG lengths varied from 38.7 cM to 124.8 cM in 'Siirt' maps. The average marker distances varied from 1.8 cM to 7.7 cM with an average of 4.6 cM. With a mean of 20.4, the number of markers in the 15 LGs ranged from 14 to 32. The number of markers per cM ranged from 0.14 to 0.57 with an average

of 0.22 in 'Siirt' female maps. In the case of the 'Pa-18' male map, 285 markers were successfully mapped to the 15 LGs. A total of 203 common markers were mapped giving a total map length of 1,362.5 cM. The number of mapped markers ranged from 14 to 28 with an average of 19. The map length ranged from 23.4 cM to 140.7 cM with an average 90.8 cM. The average marker distance ranged from 1.4 cM to 6.7 cM with an average of 4.8 cM. The average marker density was 0.21 per cM in 'Pa-18' male maps.

Comparing with the studies published by Turkeli and Kafkas (2013), Khodaeiaminjan et al. (2018), and Motalebipour et al. (2018), the current study constructed the most dense genetic linkage maps in both parents. As a result, the genetic linkage maps constructed in this current study using SNP markers will be a useful resource for future studies in QTL identification, development of molecular markers, obtaining genome sequence at the chromosomal level.

4.3. Analysis of QTLs for Leaf, Shoot and Flowering-Related Traits

4.3.1. Correlations Between Traits

Leaf length (LL), leaf width (LW), number of leaflets (NL), terminal leaflet length (TLL), terminal leaflet width (TLW), terminal leaflet shape (TLS), terminal leaflet size (TLSZ), terminal leaflet apex (TLA), terminal leaflet base (TLB), tree vigor (TV), one-year-old shoot color (OYOSC), date of first bud burst (DFBB), inflorescence abundance (IA), first bloom date (FBD), peak bloom date (PBD), last bloom date (LBD), date of vegetative bud break (DVBB), and inflorescence rachis length (IRL) of F_1 individuals derived from pistachio population 'Ohadi x Pa-18' were phenotyped during two consecutive growing seasons of 2018 and 2019. All the phenotypes showed significant variations within the F_1 population.

Leaf length (LL) with tree vigor (TV); leaf width (LW) with terminal leaflet length (TLL); terminal leaflet length (TLL) with terminal leaflet apex (TLA); terminal leaflet width (TLW) with one-year-old shoot color (OYOSC); terminal

leaflet size (TLSZ) with terminal leaflet base (TLB) and tree vigor (TV); first bloom date (FBD) with peak bloom date (PBD), last bloom date (LBD) and date of vegetative bud break (DVBB); and peak bloom date (PBD) with last bloom date (LBD) and date of vegetative bud break (DVBB) were significantly correlated ($r \geq 0.8$) in both 2018 and 2019 growing seasons (Table 4.3 and Table 4.4).

Leaf length (LL) with terminal leaflet base (TLB); leaf width (LW) with first bloom date (FBD); number of leaflets (NL) with terminal leaflet size (TLSZ), first bloom date (FBD) and date of vegetative bud break (DVBB); terminal leaflet width (TLW) with terminal leaflet size (TLSZ); inflorescence abundance (IA) with date of vegetative bud break (DVBB); and last bloom date (LBD) with date of vegetative bud break (DVBB) were significantly correlated ($r \geq 0.8$) in 2018 growing season (Table 4.3).

Leaf length (LL) with leaf width (LW) and terminal leaflet apex (TLA); leaf width (LW) with terminal leaflet size (TLSZ), terminal leaflet apex (TLA), tree vigor (TV) and date of first bud burst (DFBB); number of leaflets (NL) with terminal leaflet length (TLL); lerminal leaflet length (TLL) with tree vigor (TV) and date of first bud burst (DFBB); terminal leaflet width (TLW) with terminal leaflet shape (TLS); terminal leaflet shape (TLS) with terminal leaflet base (TLB); terminal leaflet size (TLSZ) with terminal leaflet apex (TLA); terminal leaflet apex (TLA) with tree vigor (TV) and date of first bud burst (DFBB); tree vigor (TV) with one-year-old shoot color (OYOSC), date of first bud burst (DFBB) and inflorescence abundance (IA); date of first bud burst (DFBB) with inflorescence abundance (IA); first bloom date (FBD) with inflorescence rachis length (IRL); peak bloom date (PBD) with inforescence rachis length (IRL); and last bloom date (LBD) with inflorescence rachis length (IRL) were significantly correlated ($r \geq 0.8$) in 2019 growing season (Table 4.4). All other traits showed relatively low correlation or no significant correlations between other traits (Table 4.3 and Table 4.4).

Table 4.3. The correlation among 18 different phenotypic characters related to leaf, shoot, and flowering traits in the 2018 growing season for 'Ohadi x Pa-18' F_1 population.

	LL	LW	NL	TLL	TLW	TLS	TLSZ	TLA	TLB	TV	OYOSC	DFBB	IA	FBD	PBD	LBD	DVBB	IRL
LL[1]	1																	
LW[2]	.139	1																
NL[3]	.073	.078	1															
TLL[4]	.088	**.167***	.047	1														
TLW[5]	.055	.081	.022	.089	1													
TLS[6]	.000	-.058	-.147	.069	.063	1												
TLSZ[7]	-.129	-.088	**-.157***	.076	**.157***	.076	1											
TLA[8]	-.095	-.045	.093	**-.198***	-.151	-.092	.065	1										
TLB[9]	**-.220****	-.056	.030	-.035	-.026	-.089	**.271****	.053	1									
TV[10]	**.177***	.040	.070	-.052	-.115	-.113	**-.218****	.033	-.133	1								
OYOSC[11]	-.082	.093	-.139	-.064	**.174***	.016	-.015	.047	.042	.003	1							
DFBB[12]	-.096	-.159	.012	.109	.089	.008	.126	.087	-.031	-.090	-.114	1						
IA[13]	-.047	-.040	-.144	-.117	.031	.042	-.133	.048	.027	.235	.015	-.181	1					
FBD[14]	.082	**.287***	**.285***	.022	-.009	.101	-.001	.099	.110	.105	-.076	.001	-.001	1				
PBD[15]	-.067	.083	.113	.105	-.071	.088	.147	.130	.023	-.086	-.098	.171	-.060	**.672****	1			
LBD[16]	-.148	.028	.042	.061	-.182	.105	.053	.143	.148	-.092	-.138	.138	.010	**.629****	**.903****	1		
DVBB[17]	.080	-.102	**.253***	.057	-.098	-.016	.086	.089	-.090	-.150	-.124	.222	**-.267***	**.260***	**.272***	**.254***	1	
IRL[18]	.074	-.103	.081	-.149	-.167	.043	-.108	-.050	-.049	-.037	-.037	.017	.221	.007	-.169	-.134	-.008	1

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

[1]LL: Leaf Length, [2]LW: Leaf Width, [3]NL: Number of Leaflets, [4]TLL: Terminal Leaflet Length, [5]TLW: Terminal Leaflet Width, [6]TLS: Terminal Leaflet Shape, [7]TLSZ: Terminal Leaflet Size, [8]TLA: Terminal Leaflet Apex, [9]TLB: Terminal Leaflet Base, [10]TV: Tree Vigor, [11]OYOSC: One-Year-Old Shoot Color, [12]DFBB: Date of First Bud Burst, [13]IA: Inflorescence Abundance, [14]FBD: First Bloom Date, [15]PBD: Peak Bloom Date, [16]LBD: Last Bloom Date, [17]DVBB: Date of Vegetative Bud Break, and [18]IRL: Inflorescence Rachis Length.

Table 4.4. The correlation among 18 different phenotypic characters related to leaf, shoot, and flowering traits in the 2019 growing season for 'Ohadi x Pa-18' F_1 population.

	LL	LW	NL	TLL	TLW	TLS	TLSZ	TLA	TLB	TV	OYOSC	DFBB	IA	FBD	PBD	LBD	DVBB	IRL
LL[1]	1																	
LW[2]	**.648****	1																
NL[3]	-.047	-.043	1															
TLL[4]	.147	**.212****	**-.164***	1														
TLW[5]	.089	-.088	.001	.136	1													
TLS[6]	-.027	.057	-.103	.070	**.162***	1												
TLSZ[7]	-.099	**-.181***	-.021	.030	.052	.022	1											
TLA[8]	**-.196***	**-.212****	.017	**-.241****	-.017	.095	**.168***	1										
TLB[9]	-.065	-.050	-.029	.075	-.004	**.174***	**.221****	.128	1									
TV[10]	**.239****	**.314****	-.040	**.285****	.044	-.050	**-.234****	**-.281****	-.078	1								
OYOSC[11]	-.119	-.143	.094	-.076	**-.192***	-.106	.106	.128	.082	**-.211****	1							
DFBB[12]	-.090	**-.234****	.099	**-.166***	.043	.070	-.118	**.185***	.141	**-.298****	.042	1						
IA[13]	-.045	.171	-.245	.148	-.258	-.177	.157	.045	.039	**.359****	-.021	**-.445****	1					
FBD[14]	.075	-.049	.039	-.159	.162	.144	-.051	.085	.195	-.088	-.129	-.004	-.232	1				
PBD[15]	.088	-.063	.038	-.207	.180	.088	-.047	.101	.110	-.189	-.035	-.046	-.264	**.923****	1			
LBD[16]	-.006	-.008	.042	-.181	.046	.069	-.056	.123	.040	-.200	-.035	.060	-.146	**.800****	**.899****	1		
DVBB[17]	-.128	-.046	.166	.002	-.030	-.037	.011	.092	-.015	.044	.132	-.054	.238	**.329***	**.304***	.264	1	
IRL[18]	-.097	.113	.016	.042	.003	-.175	-.112	-.062	-.206	.050	.044	.177	.245	**-.402****	**-.376****	**-.297***	.170	1

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

[1]LL: Leaf Length, [2]LW: Leaf Width, [3]NL: Number of Leaflets, [4]TLL: Terminal Leaflet Length, [5]TLW: Terminal Leaflet Width, [6]TLS: Terminal Leaflet Shape, [7]TLSZ: Terminal Leaflet Size, [8]TLA: Terminal Leaflet Apex, [9]TLB: Terminal Leaflet Base, [10]TV: Tree Vigor, [11]OYOSC: One-Year-Old Shoot Color, [12]DFBB: Date of First Bud Burst, [13]IA: Inflorescence Abundance, [14]FBD: First Bloom Date, [15]PBD: Peak Bloom Date, [16]LBD: Last Bloom Date, [17]DVBB: Date of Vegetative Bud Break, and [18]IRL: Inflorescence Rachis Length.

4.3.2. Frequency Distribution of the Morphological Traits

The frequency distribution of leaf length (LL), leaf width (LW), number of leaflets (NL), terminal leaflet length (TLL), terminal leaflet width (TLW), terminal leaflet shape (TLS), terminal leaflet size (TLSZ), terminal leaflet apex (TLA), terminal leaflet base (TLB), tree vigor (TV), one-year-old shoot color (OYOSC), date of first bud burst (DFBB), inflorescence abundance (IA), first bloom date (FBD), peak bloom date (PBD), last bloom date (LBD), date of vegetative bud break (DVBB), and inflorescence rachis length (IRL) of F_1 individuals derived from pistachio population 'Ohadi x Pa-18' were performed during two consecutive growing seasons of 2018 and 2019. The mean, maximum value, minimum value, and standard deviation of all of the traits are shown in Table 4.5.

Table 4.5. The mean, maximum value, minimum value, and standard deviation of all observed traits evaluated in the 2018 and 2019 growing seasons in the 'Ohadi x Pa-18' F_1 population.

Trait	Year	F1 Progenies			
		Mean	Max.	Min.	SD
LL[1]	2018	14.1	19.4	9	1.8
	2019	11.6	16.9	5	1.4
LW[2]	2018	10.7	15.8	6.2	1.6
	2019	8.9	12.7	2	1.3
NL[3]	2018	2.4	3.3	1.3	0.3
	2019	2.6	3.6	1.8	0.3
TLL[4]	2018	6.3	8.3	4.6	0.7
	2019	6.4	9.7	4.1	0.9
TLW[5]	2018	3.2	4.6	2.4	0.4
	2019	3.2	4.3	2.2	0.4
TLS[6]	2018	2.1	4	1	0.6
	2019	2.2	4	1	0.5
TLSZ[7]	2018	1.7	2	1	0.4
	2019	1.4	2	1	0.5
TLA[8]	2018	1.5	4	1	1.1

Trait	Year	F1 Progenies			
		Mean	Max.	Min.	SD
	2019	1.1	4	1	0.6
TLB[9]	2018	2.1	4	1	1
	2019	1.7	4	1	1
TV[10]	2018	5.4	7	3	1.3
	2019	5.1	7	3	1.6
OYOSC[11]	2018	1.8	3	1	0.6
	2019	2.2	3	1	0.7
DFBB[12]	2018	80.1	81	77	1.1
	2019	79.1	86	68	3.2
IA[13]	2018	4.3	7	3	1.4
	2019	5.8	7	3	1.6
FBD[14]	2018	89.2	93	79	2
	2019	105.4	112	101	2.7
PBD[15]	2018	91	95	88	1.4
	2019	108.6	114	104	2.7
LBD[16]	2018	92.7	98	90	1.8
	2019	112.2	117	108	2.2
DVBB[17]	2018	83.6	88	75	2.6
	2019	99.3	105	97	1.6
IRL[18]	2018	5.7	8.9	2.8	1.3
	2019	7.9	11.9	3.7	2.6

[1]LL: Leaf Length, [2]LW: Leaf Width, [3]NL: Number of Leaflets, [4]TLL: Terminal Leaflet Length, [5]TLW: Terminal Leaflet Width, [6]TLS: Terminal Leaflet Shape, [7]TLSZ: Terminal Leaflet Size, [8]TLA: Terminal Leaflet Apex, [9]TLB: Terminal Leaflet Base, [10]TV: Tree Vigor, [11]OYOSC: One-Year-Old Shoot Color, [12]DFBB: Date of First Bud Burst, [13]IA: Inflorescence Abundance, [14]FBD: First Bloom Date, [15]PBD: Peak Bloom Date, [16]LBD: Last Bloom Date, [17]DVBB: Date of Vegetative Bud Break, and [18]IRL: Inflorescence Rachis Length.

4.3.2.1. Leaf Length (LL)

The leaf length (LL) of the F_1 population 'Ohadi x Pa-18' was measured in the growing seasons of 2018 and 2019. The measurement was taken as cm (centimeter) by a ruler. The values of LL varied from 9 to 19.4 and 5 to 16.9 in 2018 and 2019, respectively. The average LL values for two years; 2018 and 2019 were 14.1 and 11.6, respectively. The standard deviation of LL values for two years was 1.8 and 1.4, respectively (Table 4.5). The frequency distribution of LL values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.16).

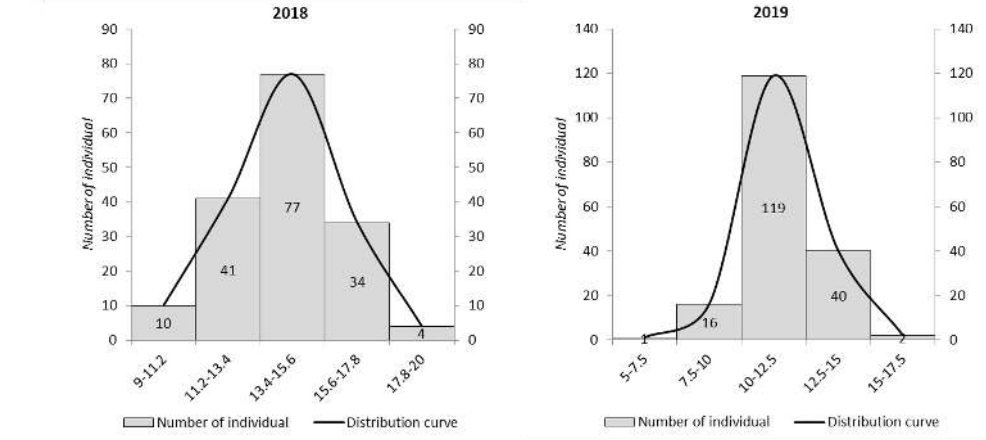

Figure 4.16. Frequency distribution of leaf length (LL) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.2. Leaf Width (LW)

The leaf width (LW) of the F_1 population 'Ohadi x Pa-18' was measured in the growing seasons of 2018 and 2019. The measurement was taken as cm by a ruler. The values of LW varied from 6.2 to 15.8 and 2 to 12.7 in 2018 and 2019, respectively. The average LW values for two years; 2018 and 2019 were 10.7 and 8.9, respectively. The standard deviation of LL values for two years was 1.6 and 1.3, respectively (Table 4.5). The frequency distribution of LW values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.17).

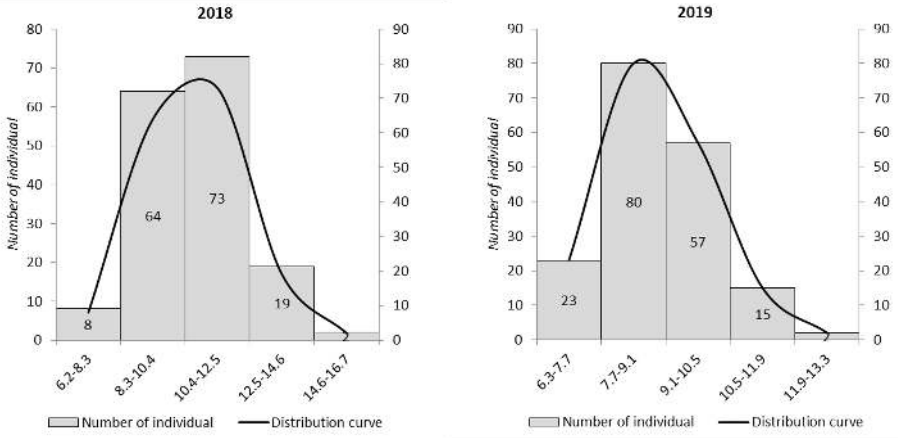

Figure 4.17. Frequency distribution of leaf width (LW) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.3. Number of Leaflets (NL)

The number of leaflets (NL) of the F_1 population 'Ohadi x Pa-18' was counted in the growing seasons of 2018 and 2019. The values of NL varied from 1.3 to 3.3 and 1.8 to 3.6 in 2018 and 2019, respectively. The average NL values for two years; 2018 and 2019 were 2.4 and 2.6, respectively. The standard deviation of NL values for both years was 0.3 (Table 4.5). The frequency distribution of NL values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.18).

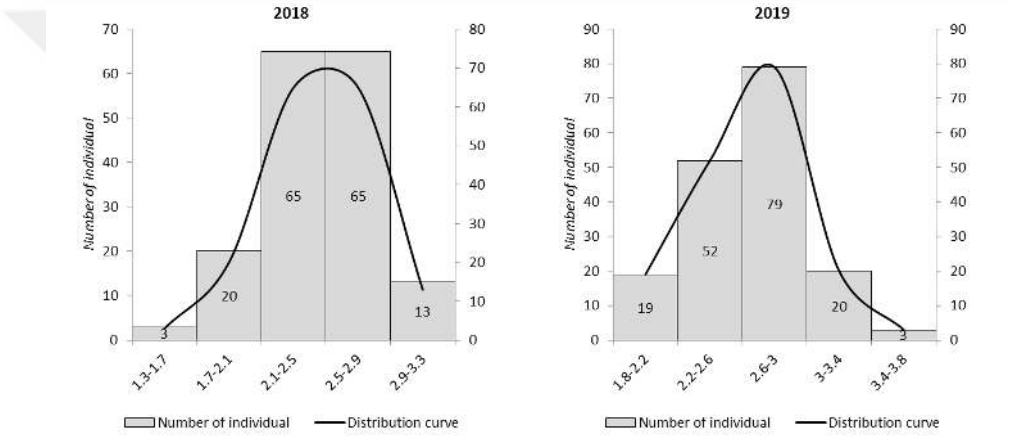

Figure 4.18. Frequency distribution of the number of leaflets (NL) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.4. Terminal Leaflet Length (TLL)

The terminal leaflet length (TLL) of the F_1 population 'Ohadi x Pa-18' was measured in the growing seasons of 2018 and 2019. The measurement was taken as cm by a ruler. The values of TLL varied from 4.6 to 8.3 and 4.1 to 9.7 in 2018 and 2019, respectively. The average TLL values for 2018 and 2019 were 0.7 and 0.9, respectively. The standard deviation of TLL values for two years was 0.7 and 0.9, respectively (Table 4.5). The frequency distribution of TLL values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.19).

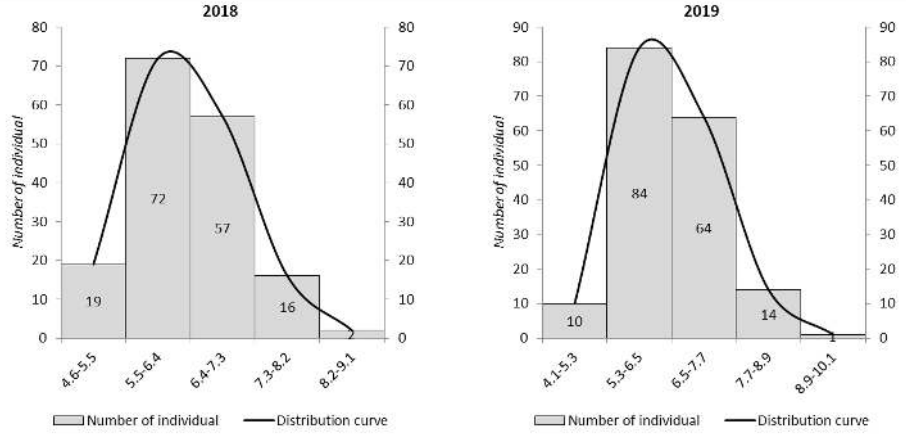

Figure 4.19. Frequency distribution of terminal leaflet length (TLL) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.5. Terminal Leaflet Width (TLW)

The terminal leaflet width (TLW) of the F_1 population 'Ohadi x Pa-18' was measured in the growing seasons of 2018 and 2019. The measurement was taken as cm by a ruler. The values of TLW varied from 2.4 to 4.6 and 2.2 to 4.3 in 2018 and 2019, respectively. The average TLW values for both years; 2018 and 2019 were the same as 3.2. The standard deviation of TLW values for both years was 0.4 (Table 4.5). The frequency distribution of TLW values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.20).

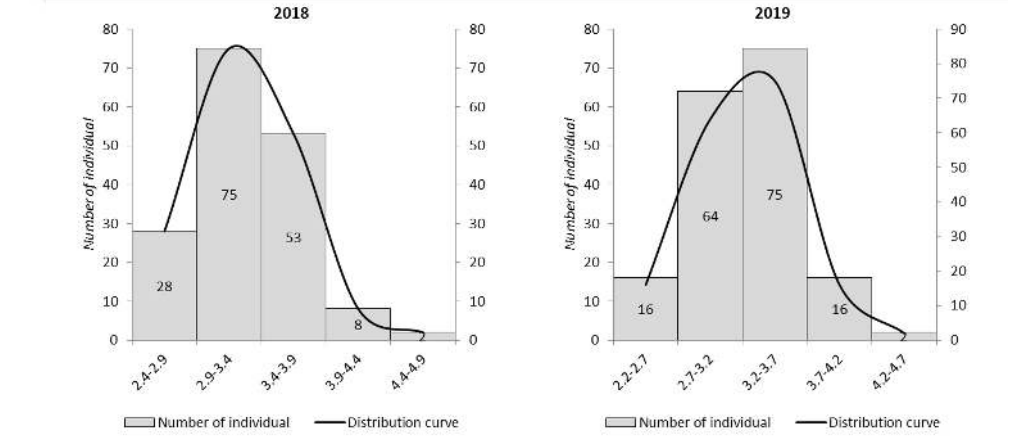

Figure 4.20. Frequency distribution of terminal leaflet width (TLW) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.6. Terminal Leaflet Shape (TLS)

The terminal leaflet shape (TLS) of the F_1 population 'Ohadi x Pa-18' was observed in the growing seasons of 2018 and 2019. The values of TLS varied from 1 to 4 both in 2018 and 2019. The average TLS values for two years; 2018 and 2019 were 2.1 and 2.2, respectively. The standard deviation of TLS values for two years was 0.6 and 0.5, respectively (Table 4.5). The frequency distribution of TLS values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.21).

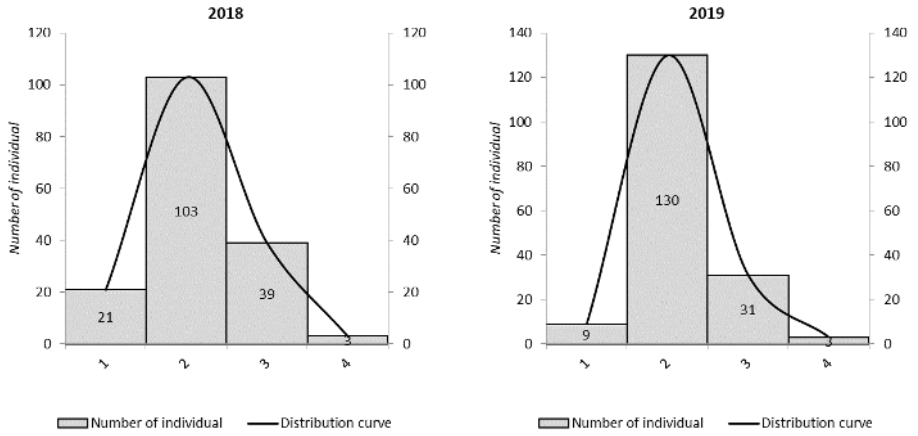

Figure 4.21. Frequency distribution of terminal leaflet shape (TLS) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.7. Terminal Leaflet Size (TLSZ)

The terminal leaflet size (TLSZ) of the F_1 population 'Ohadi x Pa-18' was observed in the growing seasons of 2018 and 2019. The values of TLSZ varied from 1 to 2 both in 2018 and 2019. The average TLSZ values for two years; 2018 and 2019 were 1.7 and 1.4, respectively. The standard deviation of TLSZ values for two years was 0.4 and 0.5, respectively (Table 4.5). The frequency distribution of TLSZ values in 2018 and 2019 gave a good fit to random distribution (Figure.4.22).

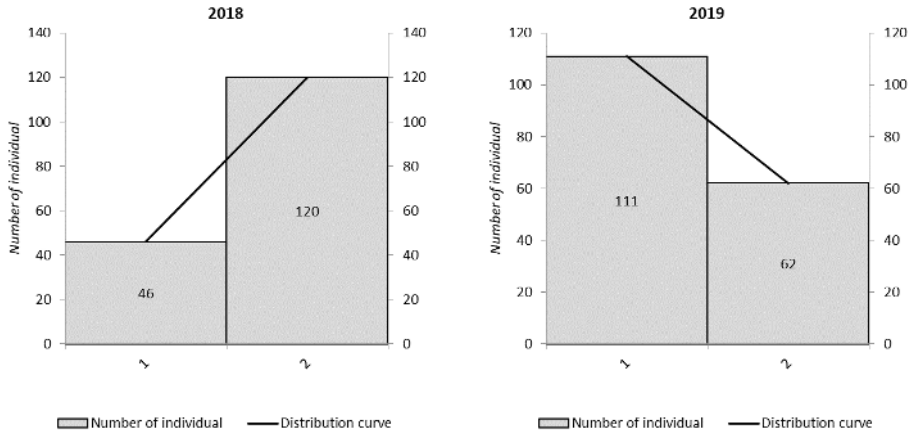

Figure 4.22. Frequency distribution of terminal leaflet size (TLSZ) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.8. Terminal Leaflet Apex (TLA)

The terminal leaflet apex (TLA) of the F_1 population 'Ohadi x Pa-18' was observed in the growing seasons of 2018 and 2019. The values of TLA varied from 1 to 4 both in 2018 and 2019. The average TLA values for two years; 2018 and 2019 were 1.5 and 1.1, respectively. The standard deviation of TLA values for two years was 1.1 and 0.6, respectively (Table 4.5). The frequency distribution of TLA values in 2018 and 2019 gave a good fit to right-skewed distribution (Figure 4.23).

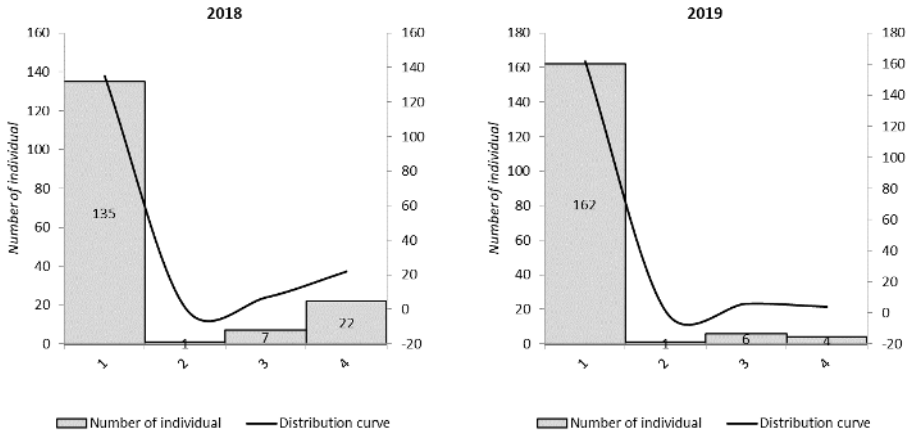

Figure 4.23. Frequency distribution of terminal leaflet apex (TLA) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.9. Terminal Leaflet Base (TLB)

The terminal leaflet base (TLB) of the F_1 population 'Ohadi x Pa-18' was observed in the growing seasons of 2018 and 2019. The values of TLB varied from 1 to 4 both in 2018 and 2019. The average TLB values for two years; 2018 and 2019 were 2.1 and 1.7, respectively. The standard deviation of TLB values for both years was the same as 1 (Table 4.5). The frequency distribution of TLB values in 2018 and 2019 gave a good fit for bi-modal distribution (Figure 4.24).

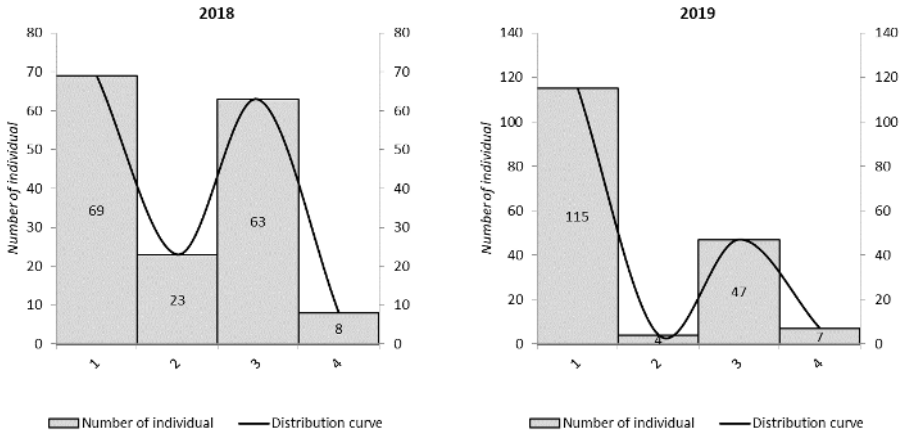

Figure 4.24. Frequency distribution of terminal leaflet base (TLB) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.10. Tree Vigor (TV)

The tree vigor (TV) of the F_1 population 'Ohadi x Pa-18' was observed in the growing seasons of 2018 and 2019. The values of TV varied from 3 to 7 both in 2018 and 2019. The average TV values for two years; 2018 and 2019 were 5.4 and 5.1, respectively. The standard deviation of TV values for two years was 1.3 and 1.6, respectively (Table 4.5). The frequency distribution of TV values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.25).

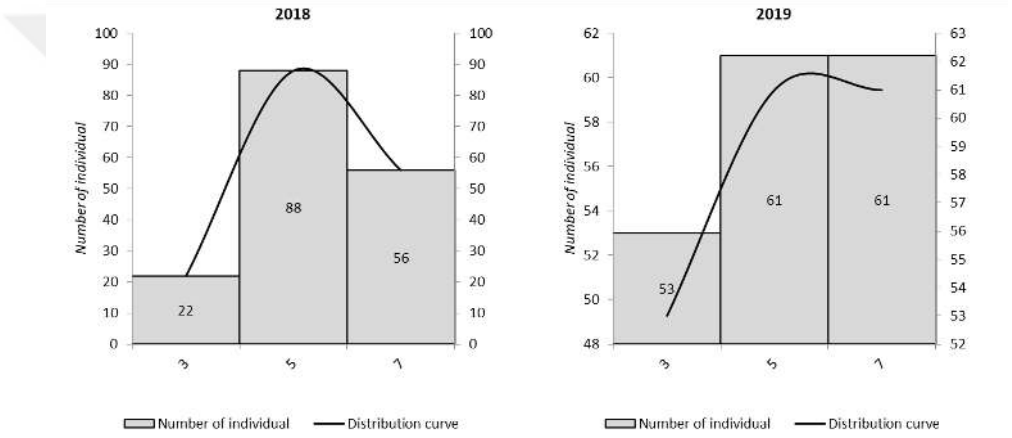

Figure 4.25. Frequency distribution of tree vigor (TV) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.11. One-Year-Old Shoot Color (OYOSC)

The one-year-old shoot color (OYOSC) of the F_1 population 'Ohadi x Pa-18' was observed in the growing seasons of 2018 and 2019. The values of OYOSC varied from 1 to 3 both in 2018 and 2019. The average OYOSC values for two years; 2018 and 2019 were 1.8 and 2.2, respectively. The standard deviation of OYOSC values for two years was 0.6 and 0.7, respectively (Table 4.5). The frequency distribution of OYOSC values in 2018 and 2019 gave a good fit to the normal distribution (Figure.4.26).

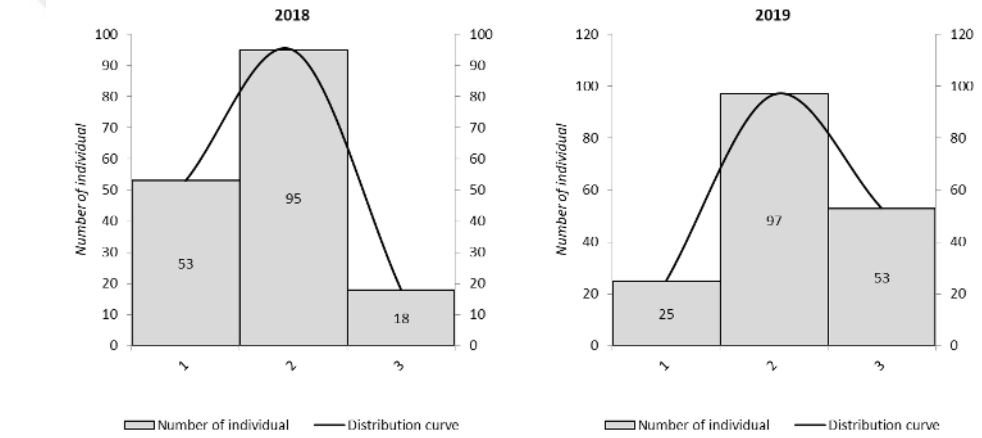

Figure 4.26. Frequency distribution of one-year-old shoot color (OYOSC) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.12. Date of First Bud Burst (DFBB)

The date of first bud burst (DFBB) of F_1 population 'Ohadi x Pa-18' was counted in the growing seasons of 2018 and 2019. The values of DFBB varied from 77 to 81 and 68 to 86 in 2018 and 2019, respectively. The average DFBB values for two years; 2018 and 2019 were 80.1 and 79.1, respectively. The standard deviation of DFBB values for two years was 1.1 and 3.2, respectively (Table 4.5). The frequency distribution of DFBB values in 2018 and 2019 gave a good fit to left-skewed distribution (Figure.4.27).

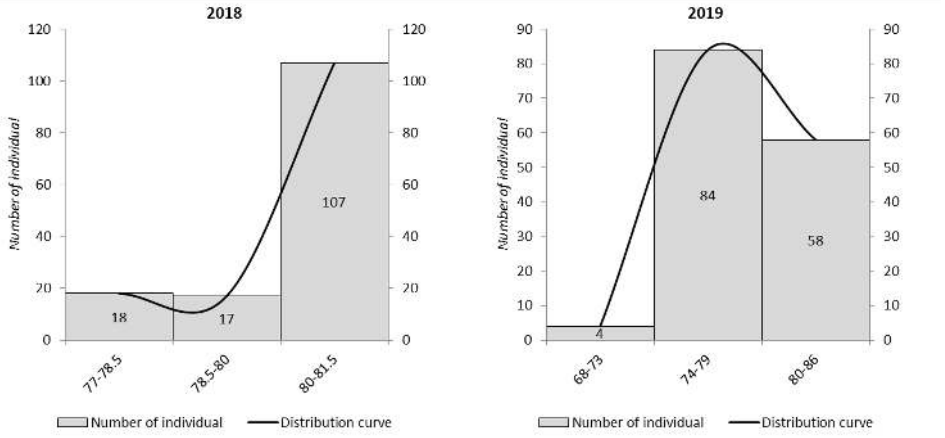

Figure 4.27. Frequency distribution of date of first bud burst (DFBB) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.13. Inflorescence Abundance (IA)

The inflorescence abundance (IA) of the F_1 population 'Ohadi x Pa-18' was observed in the growing seasons of 2018 and 2019. The values of IA varied from 3 to 7 both in 2018 and 2019. The average IA values for two years; 2018 and 2019 were 4.3 and 5.8, respectively. The standard deviation of IA values for two years was 1.4 and 1.6, respectively (Table 4.5). The frequency distribution of IA values in 2018 and 2019 gave a good fit to random distribution (Figure 4.28).

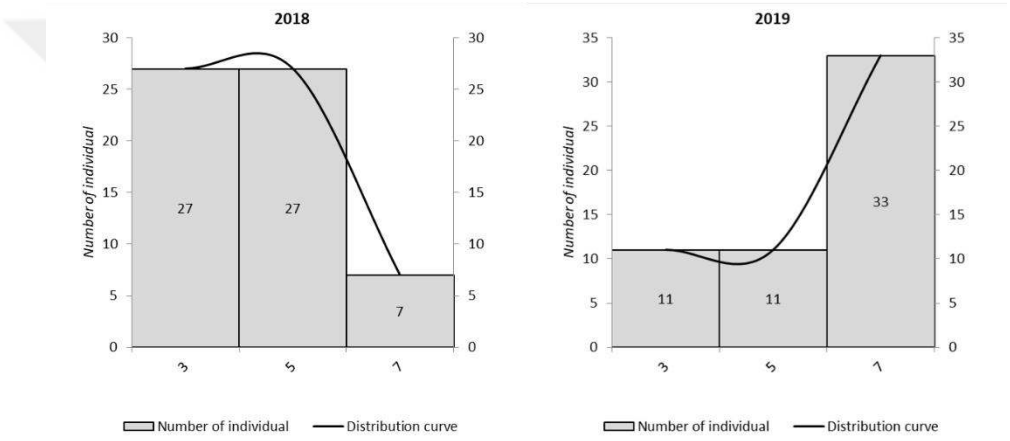

Figure 4.28. Frequency distribution of inflorescence abundance (IA) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.14. First Bloom Date (FBD)

The first bloom date (FBD) of the F_1 population 'Ohadi x Pa-18' was counted in the growing seasons of 2018 and 2019. The values of FBD varied from 79 to 93 and 101 to 112 in 2018 and 2019, respectively. The average FBD values for two years; 2018 and 2019 were 89.2 and 105.4, respectively. The standard deviation of FBD values for two years was 2 and 2.7, respectively (Table 4.5). The frequency distribution of FBD values in 2018 and 2019 gave a good fit to random distribution (Figure.4.29).

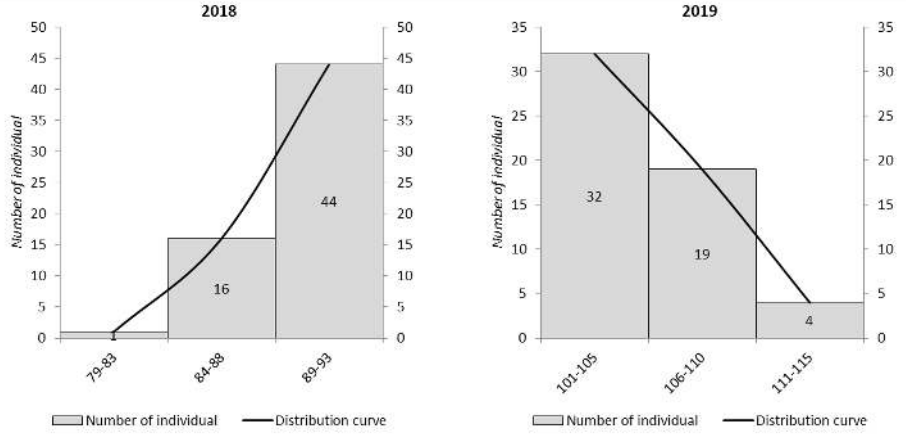

Figure 4.29. Frequency distribution of first bloom date (FBD) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.15. Peak Bloom Date (PBD)

The peak bloom date (PBD) of the F_1 population 'Ohadi x Pa-18' was counted in the growing seasons of 2018 and 2019. The values of PBD varied from 88 to 95 and 104 to 114 in 2018 and 2019, respectively. The average PBD values for two years; 2018 and 2019 were 91 and 108.6, respectively. The standard deviation of PBD values for two years was 1.4 and 2.7, respectively (Table 4.5). The frequency distribution of PBD values in 2018 and 2019 gave a good fit to right-skewed distribution (Figure.4.30).

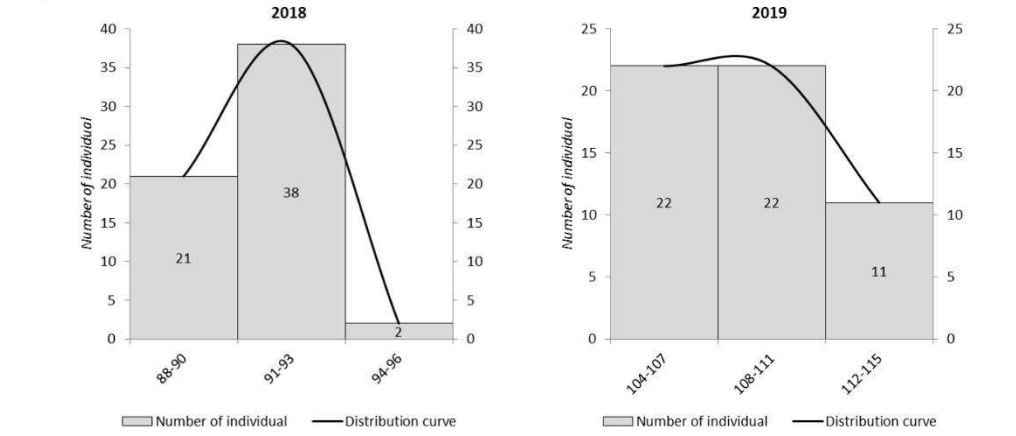

Figure 4.30. Frequency distribution of peak bloom date (PBD) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.16. Last Bloom Date (LBD)

The last bloom date (LBD) of the F_1 population 'Ohadi x Pa-18' was counted in the growing seasons of 2018 and 2019. The values of LBD varied from 90 to 98 and 108 to 117 in 2018 and 2019, respectively. The average LBD values for two years; 2018 and 2019 were 92.7 and 112.2, respectively. The standard deviation of LBD values for two years was 1.8 and 2.2, respectively (Table 4.5). The frequency distribution of LBD values in 2018 and 2019 gave a good fit to random distribution (Figure.4.31).

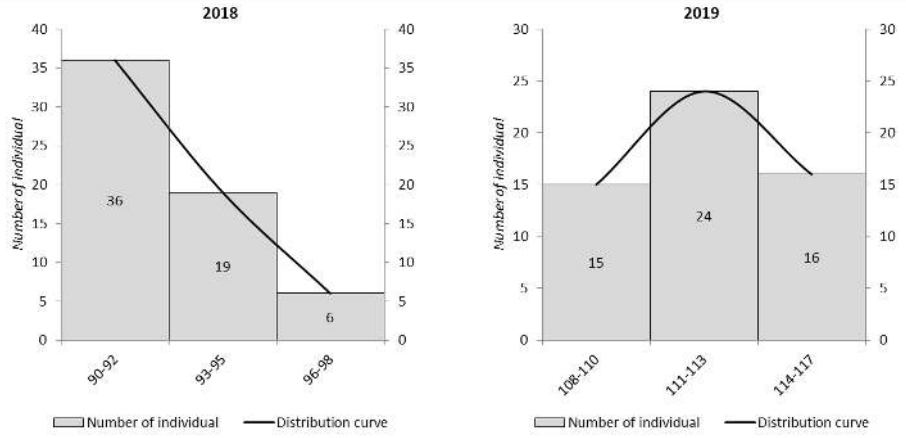

Figure 4.31. Frequency distribution of last bloom date (LBD) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.17. Date of Vegetative Bud Break (DVBB)

The date of vegetative bud break (DVBB) of the F_1 population 'Ohadi x Pa-18' was counted in the growing seasons of 2018 and 2019. The values of DVBB varied from 75 to 88 and 97 to 105 in 2018 and 2019, respectively. The average DVBB values for two years; 2018 and 2019 were 93.6 and 99.3, respectively. The standard deviation of DVBB values for two years was 2.6 and 1.6, respectively (Table 4.5). The frequency distribution of DVBB values in 2018 and 2019 gave a good fit to random distribution (Figure 4.32).

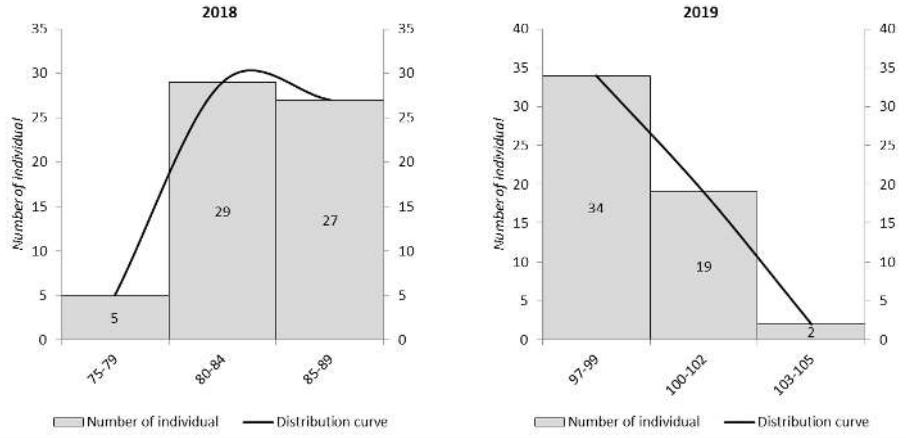

Figure 4.32. Frequency distribution of date of vegetative bud break (DVBB) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in the 2018 and 2019 growing seasons.

4.3.2.18. Inflorescence Rachis Length (IRL)

The inflorescence rachis length (IRL) of the F_1 population 'Ohadi x Pa-18' was measured in the growing seasons of 2018 and 2019. The measurement was taken in cm by a ruler. The values of IRL varied from 2.8 to 8.9 and 3.7 to 11.9 in 2018 and 2019, respectively. The average IRL values for two years; 2018 and 2019 were 5.7 and 7.9, respectively. The standard deviation of IRL values for two years was 1.3 and 2.6, respectively (Table 4.5). The frequency distribution of IRL values in 2018 and 2019 gave a good fit to the normal distribution (Figure 4.33).

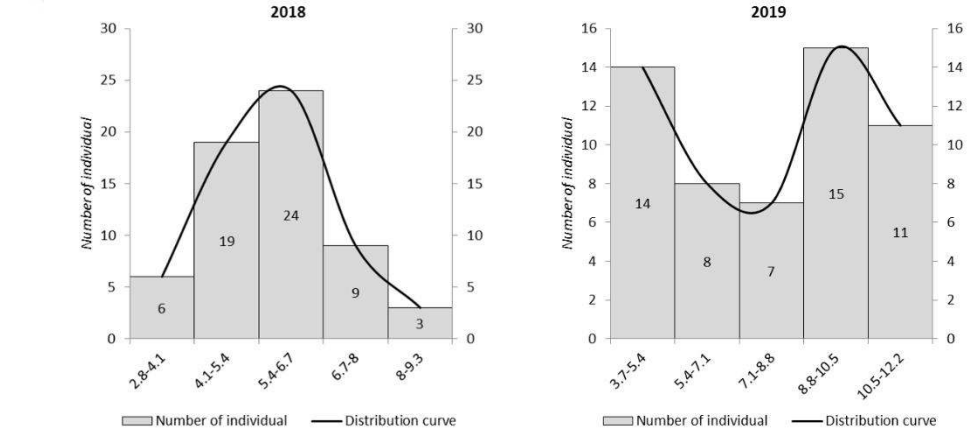

Figure 4.33. Frequency distribution of inflorescence rachis length (IRL) value in 'Ohadi x Pa-18' F_1 population. Phenotypic data were collected in 2018 and 2019 growing seasons.

4.3.3. Identification of QTLs for Leaf, Shoot and Flowering-Related Traits in 'Ohadi x Pa-18' F_1 Population

The parental maps of the 'Ohadi x Pa-18' population were used for the QTL analysis. Phenotypic data of a total of 18 traits were collected and used for QTL analysis. We found a total of four QTLs that were related to four traits and detected on three linkage groups in the 'Ohadi' map. We could not find any QTL in observed

phenotypic traits for the ‘Pa-18’ map. The QTLs that were detected at least in two of the growing seasons were considered significant (Table 4.6).

Table 4. 6. Summary of identified QTLs in 'Ohadi x Pa-18' F_1 population.

Map	Phenotype	Year	LG	GW	cM	LOD	PV%	Closest Marker	Position (cM)	Kruskal-Wallis Test Significance
Ohadi	Terminal Leaflet Base	2018	1	1.9	102.74-103.74	2.2	6	7265120	103.74	****
		2019	1	1.8	101.74-103.74	2.88	7.6	7265120	103.74	*****
	Leaf Width [cm]	2018	12	1.7	9.38-15.41	3.48	9.3	11981553	10.418	******
		2019	12	1.5	9.27-10.38	2.49	6.4	11986479	9.38	******
	Tree Vigor	2018	12	1.8	69.66-79.10	5.05	13.6	7289616	75.04	*******
		2019	12	1.7	69.66-79.10	5.89	14.9	7289616	75.04	*******
	Infloroscence Rachis Length [cm]	2018	13	1.6	29.39-30.44	2.28	15.8	7284566	30.44	***
		2019	13	1.6	30.78-30.98	4.49	31.3	7288841	30.98	******

4.3.3.1. Terminal Leaflet Base (TLB)

In the 'Ohadi x Pa-18' F_1 population, one significant QTL for the terminal leaflet base (TLB) was mapped into the tail of the LG1 of the female map in both years. No significant QTL for TLB was detected in the male map. The highest LOD scores were 2.2 and 2.9, while the highest percentage of the explained phenotypic variation was 6% and 7.6% in the female map in 2018 and 2019, respectively. The closest marker in LG1 was 7265120 (103.7 cM) in both years (Table 4.6, Figure 4.34).

4.3.3.2. Leaf Width (LW)

In the 'Ohadi x Pa-18' population, one significant QTL for leaf width (LW) was mapped into the head of LG12 of the female map in both years. No significant QTL for LW was detected in the male map. The highest LOD scores were 3.5 and 2.5, while the highest percentage of the explained phenotypic variation was 9.3% and 6.4% in the female map in 2018 and 2019, respectively. The closest marker in LG12 was 11981553 (10.4 cM) and 11986479 (9.4 cM) in 2018 and 2019, respectively (Table 4.6, Figure 4.34).

4.3.3.3. Tree Vigor (TV)

In 'Ohadi x Pa-18' population, one significant QTL for tree vigor (TV) was mapped into the tail of LG12 of the female map in both years. No significant QTL for TV was detected in the male map. The highest LOD scores were 5.1 and 5.9, while the highest percentage of the explained phenotypic variation was 13.6% and 14.9% in the female map in 2018 and 2019, respectively. The closest marker in LG12 was 7289616 (75 cM) both in 2018 and 2019 (Table 4.6, Figure 4.34).

4.3.3.4. Inflorescence Rachis Length (IRL)

In the 'Ohadi x Pa-18' population, one significant QTL for inflorescence rachis length (IRL) was mapped into the middle part of the LG13 of the female map in the year 2018 and 2019. No significant QTL for IRL was detected in the male map. The highest LOD scores were 2.3 and 4.5, while the highest percentage of the explained phenotypic variation was 15.8% and 31.3% in the female map in 2018 and 2019, respectively. The closest marker in LG13 was 7284566 (30.4 cM) and 7288841 (31 cM) in 2018 and 2019, respectively (Table 4.6, Figure 4.34).

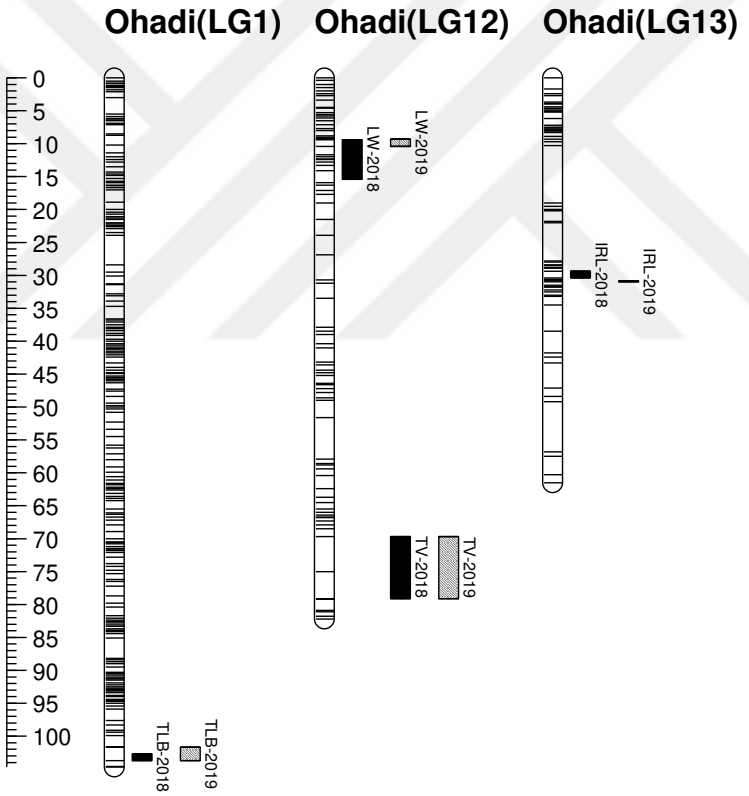

Figure 4.34. QTLs in 'Ohadi x Pa-18' F_1 population showing identified regions on LG1, LG12, and LG13 related to TLB (terminal leaflet base), LW (leaf width), TV (tree vigor), and IRL (inflorescence rachis length) in female maps for 2018 and 2019 growing seasons.

In the literature, Motalebipour et al. (2018) studied the QTL analysis in pistachio for the first tıme. In their study, the researchers used a genetic linkage map constructed by SSR markers from the crossing between *P. vera* cv. 'Siirt' and monoecious *P. atlantica* cv. 'Pa-18' F_1 population to study the QTLs about the leaf and shoot traits in 2015 and 2016 growing seasons. They identified a total of 17 QTLs in the parental maps that included: Leaf length (LL), leaf width (LW), leaf length/leaf width ratio (LWR), number of leaflet pairs (NLL), and young shoot color (YSC). Four QTLs for LL and LW were located on LG2 and LG4, while four QTLs for LWR on LG13 and LG14, two QTLs for NLL and two QTLs for YSC were on LG7 and LG9, respectively, with similar positions in both parental maps.

In this current study, an interspecific F_1 population derived from *P. vera* cv. 'Ohadi' and monoecious *P. atlantica* cv. 'Pa-18' ('Ohadi x Pa-18') were used to construct the first SNP-based genetic linkage map in pistachio and the subsequent analysis of QTLs regarding leaf, shoot, and flowering related traits in 2018 and 2019 growing seasons. There were 190 F_1 individuals taken place in this current study that is higher in number compared to Motalebipour et al. (2018)'s study where only 126 F_1 individuals were taken place. Moreover, QTL related to terminal leaflet base (TLB), tree vigor (TV), and inflorescence rachis length (IRL) in pistachio was first reported in this current study. Although the QTLs in this study were found in the maternal ('Ohadi') maps only, no significant QTLs were reported in the paternal ('Pa-18') maps. This might be occurred due to the deaths of many individuals in both growing seasons that led to a significant data loss (missing data) about the morphological traits. The missing data due to the death of individuals might also lead to limit the findings of other important QTLs in this study. The data produced in this study will be an important resource for further study in molecular breeding, development of molecular markers, and QTL analysis in pistachio and other related species in the future.

5. CONCLUSIONS

Pistachio is an important cultivated tree due to its edible nuts having beneficial properties to human health as well as commercial importance in sweetmeat industries. On the other hand, wild *Pistacia* species are widely used to produce timber, oil, and soap. Moreover, wild *Pistacia* has edible seeds and are used as rootstock sources for cultivated *P. vera*. The farmers in Turkey and in other countries paying more attention to cultivate *P. vera* due to its commercial importance. However, the alternate bearing and long juvenile period of pistachio are hampering the expected production per year that eventually requiring more production costs.

The construction of genetic linkage maps is usually a starting point of several downstream studies such as QTL, MAS, development of molecular markers, gaining a genome sequence at a chromosomal level. Different molecular marker methods such as AFLP, RAPD, RFLP, SSR, ISSR, and SNP were applied to understand the molecular mechanisms in pistachio. However, the construction of genetic linkage maps in pistachio was limited only in a few previous studies that did not eventually reveal the complete scenario of pistachio chromosomes. In our current study, we tried to overcome the drawbacks of previously published studies and to have a dense genetic linkage map using the highest numbers of SNP markers. We aimed to collect the genetic information lying inside the 15 haploid chromosomes of pistachio to switch the classic breeding into molecular breeding. To satisfy the objective, we constructed the first high-density genetic linkage maps in pistachio from an interspecific F_1 population of 'Ohadi x Pa-18' cultivars using SNP markers. The results obtained from this study are summarized below:

1. The first high-density genetic linkage maps in pistachio from an interspecific F_1 population of 'Ohadi x Pa-18' using SNP markers obtained.
2. In 'Ohadi' maternal map, a total of 1,407 SNP markers were mapped in a total map length of 1,122.2 cM with an average marker density of 1.22 per cM.
3. In the 'Pa-18' paternal map, a total of 2,518 SNP markers were mapped in a total map length of 1,307.2 cM with an average marker density of 1.93 per cM.
4. The genetic linkage maps constructed in this study were the most highly dense in pistachio to date. These highly dense genetic linkage maps are suitable to use them for QTL analysis and obtaining genome sequences at the chromosomal level.
5. A total of four significant QTLs related to terminal leaflet base, leaf width, tree vigor, and inflorescence rachis length were identified on three linkage groups (LG1, LG12, and LG13) in the 'Ohadi' map.

Turkey is one of the most important countries in the world in terms of pistachio production, however, it is far from the expected exportation of pistachio nuts. Similarly, to overcome these constraints and to switch into the molecular breeding from classic breeding, this study can be a starting point. The construction of high-density genetic linkage maps in the F_1 population of 'Ohadi x Pa-18' can be a foundation for the future QTL, and MAS studies in pistachio.

The constructed high-density genetic linkage maps of 'Ohadi x Pa-18' in this study can be used as a foundation to construct linkage maps in other populations and with other marker systems. Also, using these linkage maps along with the genomic database, it can be possible to reveal the DNA sequences at a chromosomal level in pistachio.

The constructed linkage maps in this study along with the QTL studies will allow developing markers for early selection that can be used in the molecular breeding programs. The MAS plays an important role in perennial trees especially in those with long juvenile periods like pistachio. As a result, this study provides a solid foundation for further molecular studies such as QTL analysis, marker development in pistachio.

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CURRICULUM VITAE

I was born on 15 February 1984 in Bogra, Bangladesh. I got my primary, secondary, and higher secondary education with distinctions in Bogra. In 2004, I moved to Dhaka, Bangladesh and get admitted to my undergraduate study. I completed my BS degree in Molecular Medicine and Bioinformatics from the Faculty of Life Sciences, University of Development Alternative in 2008 with the highest CGPA (3.94 on a scale of 4.00) in the department and got the Chancellor's Gold Medal award for the outstanding academic achievements. I obtained an MS degree in Biotechnology and Genetic Engineering from the Faculty of Life Sciences, University of Development Alternative in 2010 with a CGPA 3.75/4.00. In 2011, I achieved a BEd degree from the Faculty of Education, Darul Ihsan University with a CGPA 3.57/4.00. I completed an MEd degree from the Faculty of Education, Darul Ihsan University in 2013 with a CGPA of 3.48/4.00. In 2014, I got a Professional MBA degree from the M.H. School of Business, Presidency University with a CGPA 3.70/4.00. Then in 2014, I moved to Adana, Turkey with a fully-funded fellowship from the Scientific and Technological Research Council of Turkey (TÜBİTAK) to pursue my PhD in Biotechnology. I learned the Turkish language up to the C1 level as per the Common European Framework of Reference for Languages (CEFRL) and got my Diploma in the Turkish Language from Çukurova University TÖMER (Turkish Learning, Application and Research Center) in 2015. After that, I started my PhD education and research in the field of Biotechnology under the Institute of Natural and Applied Sciences, Çukurova University and completed it in 2020 with a CGPA 4.00/4.00. I have contributed to 16 full-length research and review articles and 5 conference proceedings to date. I have participated in several summer and winter schools, conferences and symposiums both at home and abroad. I have a strong command in the English language and I can be easily adopted in cross-cultural environments.