ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCE

PhD THESIS

Aibibula PAIZILA

CONSTRUCTION OF HIGH-DENSITY GENETIC LINKAGE MAPS AND QTL ANALYSIS IN ALMOND

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 the Doctor of Philosophy (PhD) by the board of Juries on 06/03/2020.

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ABSTRACT

PhD THESIS

CONSTRUCTION OF HIGH-DENSITY GENETIC LINKAGE MAPS AND QTL ANALYSIS IN ALMOND

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Almond (*Prunus dulcis* (Mill) D.A. Webb) is the only nut tree species in Rosaceae family and it is one of the most important nut crops in Turkey. Cultivar breeding programs in almond take a long time due to its long juvenile period. So, it is necessary to use molecular approach to overcome such problems. Construction of genetic linkage maps and quantitative trait locus (QTLs) analysis are important tools to develop markers linked to economically important phenotypic characters, which can significantly shorten the breeding efficiency. Therefore, in this study, we aimed (1) to construct a high-density genetic linkage map and (2) to detect QTLs associated with economically important phenotypic characters in almond. 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' F1 populations were used in genetic linkage mapping and QTL analysis. The phenotypic data obtained from 2015, 2016, 2017 and 2018 growing seasons and from 21 characters were used. SSR, DArT and SNP markers were used for high-density genetic linkage map construction. In 'Gülcan-2 x Lauranne' population, the Gülcan-2 maternal linkage map included 3,338 markers, and the total map length was 469.86 cM with 7.1 marker density (marker/cM), whereas the Lauranne male genetic map contained 3,442 markers, and the total map length was 572.27 cM with 6.23 marker density. In 'Guara x Nurlu' population, the Guara maternal linkage map included 2,563 markers, and total map length was 443.92 cM with 5.79 marker density (marker/cM), whereas the Nurlu male genetic map contained 3,135 markers, and the total map length was 516.02 cM with 6.14 marker density. QTL analysis was conducted using genetic linkage maps and phenotypic data analysis. As a result, a total of 12, 11, 23 and 22 major QTLs were detected in Gülcan-2, Lauranne, Guara and Nurlu maps, respectively. The data produced in this study will be very useful for breeding and genetic studies in the future for almond.

Key words: Almond, SSR, SNP, genetic mapping, QTL

ÖZ

DOKTORA TEZİ

BADEMDE YÜKSEK YOĞUNLUKTA GENETİK HARİTANIN OLUŞTURULMASI VE QTL ANALİZİ

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Badem (*Prunus dulcis* (Mill) D.A. Webb), Rosaceae familyasındaki tek sert kabuklu meyve türüdür ve Türkiye için önemli sert kabuklu meyvelerinden biridir. Badem çeşit ıslah çalışmaları gençlik kısırlığı sebebiyle uzun zaman almaktadır. Bu nedenle, bu gibi sorunların üstesinden gelebilmek için moleküler çalışmaların yapılmasına ihtiyaç vardır. Genetik bağlantı haritaları ve kantitatif özellik lokus analizleri, ekonomik öneme sahip ve ıslah verimliliğini artıran fenotipik karakterlerle ilişkili markör geliştirmek için oldukça önemli araçlardır. Bu çalışmanın amacı 1) bademde yüksek yoğunlukta genetik haritalar oluşturmak ve 2) ekonomik olarak önemli fenotipik karakterlerle ilgili QTL bölgelerini belirlemektir. Genetik haritalama ve QTL analizlerinde 'Gülcan-2 x Lauranne' ve 'Guara x Nurlu' populasyonları kullanılmıştır. QTL analizlerinde 2015, 2016, 2017 ve 2018 yıllarına ait 21 adet fenotipik karakterden elde edilen veriler kullanılmıştır. SSR, DArT ve SNP markörleri yoğun genetik haritalar oluşturmak için kullanılmıştır. 'Gülcan-2 x Lauranne' populasyonunda; Gülcan-2 ana genetik haritası toplam 3,338 marköre, 469.92 cM harita uzunluğuna ve 7.1 markör yoğunluğuna sahip olurken, Lauranne baba haritası ise 3,442 marköre, 6.23 markör yoğunluğuna ve 572.27 cM harita uzunluğuna sahip olmuştur. 'Guara x Nurlu' populasyonunda; Guara ana haritasında 2,563 markör, 443.92 cM toplam harita uzunluğuna ve 5.79 markör yoğunluğuna sahip olurken, Lauranne baba haritası ise 3,135 markör ile 6.14 yoğunluğa ve toplam 516.02 cM harita uzunluğuna sahip olmuştur. QTL analizlerinde sonucunda Gülcan-2, Lauranne, Guara ve Nurlu genetic haritalarında sırasıyla 12, 11, 23 ve 22 fenotipik karakterler ile ilişkili QTL bölgeleri belirlenmiştir. Sonuç olarak, bu çalışma kapsamında elde edilen veriler bademde gelecekte yapılacak olan ıslah ve genetik çalışmalar için oldukça yararlı olacaktır.

Anahtar Kelimeler: Badem, SSR, SNP, Genetik haritalama, QTL

EXTENDED ABSTRACT

Almond (*Prunus dulcis* (Mill)) D.A. Webb) is the only nut crop belongs to the Prunoideae subfamily of Rosaceae. It has a haploid chromosome number of n=8. Almond is originated in Central and Southwest Asia, then spread to the other parts of the world with carried seeds by caravans on the old Silk Road. After several centuries of expansion now almond is cultivated both in Northern and Southern hemisphere. Almond has a very high nutritional value and health benefits. That makes almond commercially one of the most important nuts, thus the production of almond is increasing year by year.

As being one of the origins of almond Turkey has a strong potential in almond production. Turkey has a suitable climate condition and land conditions for almond production. However, the yield of almond in Turkey is not satisfying due to the choice of improper cultivars. Most of the local and foreign cultivars grown in Turkey were self-incompatible and early blooming cultivars. New cultivars with late-blooming, self-compatible characteristics and also highly adaptable to local growing conditions are urgently needed in Turkey. Thus breeding of new almond cultivars becomes urgent in Turkey in order to increase the almond production efficiently.

Blooming date, self-compatibility, tree architecture, ripening time, harvest time and kernel quality are the most important objectives in almond breeding programs. Conventional plant breeding has a very long history but in perennial fruit trees like almond conventional breeding takes extremely long time due to the long juvenile period. Nowadays, breeders combine the conventional breeding and molecular breeding methods in plant breeding programs, Molecular breeding methods make plant breeding more efficient, cost effective and produces high-quality varieties in a relatively shorter period with low cost. Marker Assisted Selection (MAS) is a key step to improving the efficiency of the fruit-breeding programs. So it is very important in almond breeding programs to develop DNA

markers that closely linked to the important agronomical traits for shortening the breeding period and cutting the expenses in breeding programs.

The polygenic nature of the many important agronomical traits makes it necessary to conduct quantitative trait loci (QTL) analysis for identifying candidate genes or markers associated with important agronomical traits. High density genetic linkage maps are needed for the accurate detection of the markers that closely linked to the important trait in QTL analysis. Till today, a few genetic linkage maps had been constructed in almond using different marker systems such as isoenzyme, Random amplified polymorphic DNA (RAPD) markers, Restriction fragment length polymorphisms (RFLPs) markers, amplified fragment length polymorphisms (AFLPs) markers and single sequence repeats (SSRs) markers. However, all of the linkage maps constructed till date were low-density linkage maps, thus makes it quite hard to accurately detect the markers that closely related to the QTL region of interest.

In recent years, with the advances in next-generation sequencing technologies, new genotyping methods emerged to meet the demands for fast and cost-effective genotyping and sequencing in the plant genome. Such as restriction site-associated DNA sequencing (RAD-seq) and genotyping-by-sequencing (GBS) and Diversity Arrays Technology sequencing (DArT-seq). Among them, DArT-seq represents a new implementation of sequence reduced representations. This method uses GBS as a platform to offers affordable genome profiling which generates high-density SNPs and silico DArT (presence/absence of restriction fragments in representation) markers. There are many high-density linkage mapping and QTL analysis studies in different *Prunus* species such as; sweet cherry, peach, Japanese plum, and sour cherry. However, these types of high-density linkage mapping studies are very limited in almond.

In this study, we used the previously constructed genetic linkage maps in ‘Gülcan-2 x Lauranne’ and ‘Guara x Nurlu’ populations by SSR markers as framework linkage map. And enriched these linkage maps with large amount of

SNPs and DArT markers developed by the DArT-seq technology. And conducted QTL analysis with the four years of morphologic data related to the important nut and kernel traits in the F1 progenies of the 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' almond populations.

In this study, four parental genetic linkage maps were constructed by using SSR, SNPs and DArT markers in 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations. In 'Gülcan-2 x Lauranne' population, the Gülcan-2 maternal linkage map included 3,338 markers, and the total map length was 469.86 cM with 7.1 marker density (marker/cM), whereas the Lauranne male genetic linkage map contained 3,442 markers, and the total map length was 572.27 cM with 6.23 marker density. In 'Guara x Nurlu' population, the Guara maternal linkage map included 2,563 markers, and total map length was 443.92 cM with 5.79 marker density (marker/cM), whereas the Nurlu male genetic map contained 3,135 markers, and the total map length was 516.02 cM with 6.14 marker density. In all four genetic linkage maps all the markers were distributed along the eight linkage groups same as the haploid chromosome number of almond.

In this study, quantitative trait locus (QTL) analysis were also conducted on 21 kernel and nut related traits: Shell L (SL), Shell a (Sa), Shell b (Sb), Kernel L (KL), Kernel a (Ka), Kernel b (Kb), Nut Length (NLh), Nut Width (NW), Nut Thickness (NT), Nut Weight (NWt), Kernel Length (KLh), Kernel Width (KW), Kernel Thickness (KT), Kernel Weight (KWt), Kerbel Nut Ratio (KNR), Double Kernel Ratio (DKR), Softness of Shell (SoS), Nut Shape (NS), Shell Color Intensity (SCI), Kernel Color Intensity (KCI) and Shriveling of Kernel (SoK) in 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations. QTL analysis was conducted using four years of phenotypic data in each parental maps separately. A total of 12, 11, 23 and 22 major QTLs related to 9, 9, 15, 14 traits were detected in Gülcan-2, Lauranne, Guara and Nurlu genetic linkage maps, respectively.

The QTL of Shell L was located on LG4 in Guara map. The QTL of Shell a was located on LG4 of Gülcan-2 and Lauranne maps. The QTL of Kernel a, Kernel

Weight and Kernel Color were located on LG8 in Guara and Nurlu maps. QTL of Nut Length was located on LG2 and LG6 in Gülcan-2 and Lauranne maps, while mapped on LG7 and LG8 in Guara and Nurlu maps. The QTL of Nut Width was mapped on LG2 in Gülcan-2 and Lauranne maps, while located on LG6 in Guara and Nurlu maps. The QTL of Nut Thickness was locate on LG6 in Guara and Nurlu maps. The QTL of Nut Weight was located on LG2 and LG5 in Gülcan-2 and Lauranne maps, while mapped on LG2 in Guara map and LG6 in both Guara and Nurlu maps. The QTL of Kernel Length was located on LG6 in all four maps and mapped on LG8 in Guara and Nurlu maps. The QTL of Kernel Width was mapped on LG2 and LG6 in Guara and Nurlu maps. The QTL of Kernel Thickness was located on LG4 in Gülcan-2 and Lauranne map while mapped on LG8 in Guara and Nurlu maps.

The QTL of Kernel Nut Ratio was located on LG6 and LG8 in Guara and Nurlu maps. The QTL of Softness of Shell was mapped on LG4 in Gulacan2 and Lauranne maps. The QTL of Nut Shape was located on LG2 and LG7 in Guara and Nurlu maps. The QTL of Shriveling of Kernel was located on LG6 in Gülcan-2 and Lauranne maps while mapped on LG8 in Guara and Nurlu maps. The QTL of Double Kernel Ratio was mapped on LG1 and LG6 in Gülcan-2 and Lauranne maps, while located on LG1, LG6 and LG8 in Guara and Nurlu maps.

As a result, in this study, 1) four high-density linkage maps were constructed by using SSR, SNPs and silico DArT markers. 2) QTL analysis of 21 nut and kernel related traits was conducted in four parental linkage maps separately. These new well-saturated almond linkage maps present valuable sets of newly identified SNPs and silico DArT markers, these markers also provide a powerful tool for QTL identification and further breeding applications. The genetic linkage maps constructed in this study provides almond genomic information for the international community. Some of the closely linked markers to important agronomical traits developed in this study can be putative molecular markers that can be used in the future almond breeding studies.

GENİŞLETİLMİŞ ÖZET

Badem (*Prunus dulcis* (Mill)) D.A. Webb) Rosaceae familyasının Prunoideae alt familyasına ait olan tek sert kabuklu meyve türüdür. Bademin haploit kromozom sayısı n=8'dir. Bademin ana vatanı orta ve güneybatı Asya'dır ve badem yetiştiriciliği yüzyıllar içerisinde dünyanın dört bir yanına dağılmıştır. Badem yüksek oranda besin değerine sahip olup insan sağlığı için oldukça önemlidir. Bundan dolayı bademin hem ekonomik değeri hem de üretim miktarı gün gittikçe artmaktadır.

Türkiye bademin ana vatanlarından biri olduğu için üretimde yüksek potansiyele sahiptir ve Türkiye'nin iklim koşulları badem üreticiliği için oldukça uygundur. Ancak Türkiye'de yetiştiriciliği yapılan yerli ve yabancı çeşitlerin çoğu kendine verimsiz ve erken çiçek açan çeşitler olduğundan dolayı üretim miktarı düşük kalmıştır. Bu yüzden hem kendine uyuşan hem de geç çiçek açan aynı zamanda da toprak ve ekolojik koşullarına uyum sağlayan çeşitlerin geliştirilmesi önem arz etmektedir.

Çiçeklenme zamanı, kendine uyuşma durumu, ağaç şekli, meyve olgunlaşma zamanı ve meyve kalitesi badem ıslah programlarında önemli seçim kriterleridir. Bitkilerin ıslahında genelde geleneksel yöntemler uygulanmıştır ama çok yıllık meyve türlerinde gençlik kısırlık süresinin uzun olmasından dolayı geleneksel ıslah çalışmaları çok uzun zaman almaktadır. Son zamanlarda, ıslahçılar geleneksel bitki ıslahı ile modern moleküler ıslahı birleştirerek yürütmektedirler. Moleküler bitki ıslahı çok daha kısa bir süre içerisinde daha az maliyet ile daha fazla çeşit geliştirme imkânı sunmaktadır. Marköre dayalı seleksiyon (MAS) bitki ıslahını daha verimli duruma getirmek açısından büyük önem taşımaktadır. Dolayısıyla badem ıslah çalışmalarında önemli özelliklerle ilgili moleküler markörlerin geliştirilmesi hem ıslah süresini kısaltmak hem de maliyeti düşürmek açısından oldukça önemlidir.

Tarımsal açıdan önemli olan özelliklerin çoğu çok gen tarafından kontrol edilmektedir. Bu nedenle kantitatif özellik lokus (QTL) çalışmaları önemli özelliklerle ilişkili gen ya da markör bulmak için çok önemlidir. Genetik bağlantı haritalarının oluşturulması QTL çalışmalarının alt yapısını oluşturmaktadır. Bugüne kadar bademde birçok farklı markör sistemleri kullanılarak genetik bağlantı haritaları oluşturulmuştur (izoenzim, RFLP, RAPD, AFLP ve SSR). Ancak bu genetik haritaların hepsi düşük yoğunlukta bağlantı haritaları olup istediğimiz önemli QTL bölgesine yakın olan markör belirlemek için yetersiz kalmaktadır.

Son yıllarda yeni nesil sekanslama (NGS) teknolojilerin gelişmesiyle, bitki genomlarında hızlı ve düşük maliyet ile sekanslama ihtiyacını karşılamak için yeni genotiplendirme metotları ortaya çıkmıştır. DArT-seq yöntemi ise genotiplendirme ile sekanslama (GBS) platformu kullanarak düşük maliyetle yüksek miktarda SNP ve silico DArT (kesim enzim kesme bölgesinin var/yok olması) markörleri geliştirebilen genom profilleme yöntemidir. Kiraz, vişne, şeftali ve erik gibi farklı *Prunus* türlerinde yüksek yoğunlukta genetik bağlantı haritaları oluşturulmuştur. Ancak bu tür çalışmalar bademde oldukça sınırlı bulunmaktadır.

Bu çalışmada daha önceden SSR markörleri ile oluşturulan 'Gülcan-2 x Lauranne' ve 'Guara x Nurlu' badem populasyonlarının genetik bağlantı haritaları iskelet harita olarak kullanılmıştır. Çalışmanın devamında DArT-seq teknolojisi kullanılarak elde edilen SNP ve DArT markörleri ile yüksek yoğunlukta genetik bağlantı haritaları elde edilmiş ve bu haritaları kullanarak meyve özellikleri ile ilgili QTL bölgeleri tespit edilmiştir.

Bu çalışmada, 'Gülcan-2 x Lauranne' ve 'Guara x Nurlu' populasyonlarında SSR, SNP ve DArT markörleri kullanılarak dört farklı genetik bağlantı haritası oluşturulmuştur. 'Gülcan-2 x Lauranne' populasyonunda, Gülcan-2 anne haritasına toplam 3,338 markör haritalanmıştır ve toplam harita uzunluğu 469.9 cM, markör yoğunluğu ise 7.1 (markör/cM) olmuştur, Lauranne genetik haritasında ise 3,442 markör haritalanmış ve toplam harita uzunluğu 572.3 cM, markör yoğunluğu ise 6.2 olmuştur. 'Guara x Nurlu' populasyonunda, Guara anne haritasına toplam 2,563

markör haritalanmış ve toplam harita uzunluğu 443.9 cM, markör yoğunluğu ise 5.8 (markör/cM) olarak belirlenmiş, Nurlu genetik haritasında ise 3,135 markör haritalanmış ve toplam harita uzunluğu 516.0 cM, markör yoğunluğu ise 6.1 olarak hesaplanmıştır.

Bu çalışmada, 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' badem populasyonlarında toplam 21 adet meyve özellikleri ile ilgili kantitatif özellik lokus çalışması yapılmıştır. Bu özellikler ise: kabuk L (SL), kabuk a (Sa), kabuk b (Sb), meyve iç L (KL), meyve iç a (Ka), meyve iç b (Kb), kabuklu meyve uzunluğu (NLh), kabuklu meyve en (NW), kabuklu meyve kalınlığı (NT), kabuklu meyve ağırlığı (NWt), meyve iç uzunluğu (KLh), meyve iç en (KW), meyve iç kalınlığı (KT), meyve iç ağırlığı(KWt), randıman (KNR), çift meyve oranı (DKR), kabuk sertliği (SoS), kabuk şekli (NS), kabuk renği (SCI), meyve iç renği (KCI) ve meyve iç buruşukluluk (SoK). QTL analizi dört seneye ait fenotipik veriler kullanılarak yapılmıştır. Gülcan-2, Lauranne, Guara ve Nurlu bağlantı haritalarında sırasıyla 9, 9, 15 ve 14 tane özellik ile ilgili toplam 12, 11, 23 ve 22 tane önemli QTL bölgeleri tespit edilmiştir.

Kabuk L ile ilgili QTL bölgesi Guara haritasında LG4'de belirlenmiştir. Shell a ile ilgili özellik Gülcan-2 ve Lauranne haritalarında LG4'de yer almıştır. Meyve iç a ve Meyve iç ağırlığı ile ilgili QTL bölgesi Guara ve Nurlu haritasında LG8'de belirlenmiştir. Kabklu meyve uzunlğu ile ilgili QTL bölgesi Gülcan-2 ve Lauranne haritasında LG2'de belirlenmiştir, Guara ve Nurlu haritasında ise LG7 ve LG8' de yer almıştır. Kabuklu meyve eni ile ilgili QTL bölgesi Gülcan-2 ve Lauranne haritasında LG2'de belirlenmiştir, Guara ve Nurlu haritasında ise LG6' da yer almıştır. Kabuklu meyve kalınlığı ile ilgili QTL bölgesi Guara ve Nurlu haritasında ise LG6' da yer almıştır. Kabuklu meyve ağırlığı ile ilgili QTL bölgesi Gülcan-2 ve Lauranne haritasında LG2 ve LG5'de belirlenmiştir, Guara haritasında LG2' de, Guara ve Nurlu haritasında ise LG6' da yer almıştır. Meyve uzunluğu ile ilgili QTL bölgesi tüm haritalarda LG6'de belirlenmiştir, Guara ve Nurlu haritasında ise LG8' da yer almıştır. Meyve eni ile ilgili QTL bölgesi Guara ve Nurlu haritasında

LG2 ve LG6' dA belirlenmiştir. Meyve kalınlığı ile ilgili QTL bölgesi Gülcan-2 ve Lauranne haritasında LG4' de belirlenmiştir, Guara ve Nurlu haritasında ise LG8' de yer almıştır.

Meyve randımanı ile ilgili QTL bölgesi Guara ve Nurlu haritasında LG6 ve LG8'de belirlenmiştir. Kabuk sertliği ile ilgili QTL bölgesi Gülcan-2 ve Lauranne haritasında LG4' de belirlenmiştir. Kabuk şekli ile ilgili QTL bölgesi Guara ve Nurlu haritasında LG2 ve LG7' de belirlenmiştir, Guara ve Nurlu haritasında ise LG8' de yer almıştır. Meyve burşukluluğu ile ilgili QTL bölgesi Gülcan-2 ve Lauranne haritasında LG6' de belirlenmiştir, Guara ve Nurlu haritasında ise LG8' de yer almıştır. Çift meyve oranı ile ilgili QTL bölgesi Gülcan-2 ve Lauranne haritasında LG1 ve LG6' da belirlenmiştir, Guara ve Nurlu haritasında ise LG1, LG6 ve LG8' de yer almıştır.

Sonuç olarak, bu çalışmada 1) bademde SSR, SNPs ve silico DArT markörleri kullanılarak dört farklı yüksek yoğunluktaki genetik bağlantı haritası oluşturulmuş, 2) meyve özellikleri ile ilgili toplam 21 adet özellik için QTL analizi yapılmış ve QTL bölgeleri belirlenmiştir. Bu çalışma kapsamında çok sayıda SNPs ve silico DArT geliştirilmiştir ve bu markörleri kullanılarak oluşturulan yüksek yoğunluktaki genetik bağlantı haritaları daha sonraki badem ve yakın türlerde yapılacak olan ıslah çalışmalarında QTL ve genom çalışmaları için büyük önem arz etmektedir. Bu çalışma kapsamında belirlenen QTL bölgeleri validasyon sonrasında gelecekteki badem ıslah çalışmalarında kullanılabilecektir.

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LIST OF ABBREVIATIONS

cM	: centiMorgan
CTAB	: Cetyl Trimethyl Ammonium Bromide
dNTP	: Deoxy- Nucleoside Triphosphate
dATP	: Deoxyadenosine Triphosphate
dCTP	: Deoxycyitidine Triphosphate
dGTP	: Deoxyguanosine Triphosphate
dTTP	: Deoxythymidine Triphosphate
DNA	: Deoxyribonuleic acid
EDTA	: Etilen Diamin Tetra Asetic Acid
TE	: Tris-EDTA
g	: Gram
HCl	: Hidrochloric Acid
LOD	: Logarithm Odd Density
LG	: Linkage Group
M	: Molar
$MgCl_2$	: Magnesium Chloride
ml	: Mililitre
mM	: Milimolar
NaCl	: Sodium Chloride
$Na_2S_2O_5$	: Sodium Metabisulfite
ng	: Nanogram
PCR	: Polymerase Chain Reaction
rpm	: Revolutions per minute
AFLP	: Amplified Fragment Length Polymorphism
SNP	: Single Nucleotide Polymorphism
SSR	: Simple Sequence Repeat
RFLP	: Restriction fragment length polymorphism

RAPD : Random Amplified Polymorphic DNA

ISSR : Inter- Simple Sequence Repeat

SNP : Single-nucleotide polymorphism

DArT : Diversity Arrays Technology

MAS : Marker Assisted Selection

STS : Sequence-Tagged Sites

QTL : Quantitative Trait Loci

Taq : Thermus aquaticus

TBE : Tris-Boric acid/EDTA

µM : Micro Molar

µl : Microliter

ABI : Applied Biosystems

SL : Shell L

Sa : Shell a

Sb : Shell b

KL : Kernel L

Ka : Kernel a

Kb : Kernel b

NLh : Nut Length

NW : Nut Width

NT : Nut Thickness

NWt : Nut Weight

KLh : Kernel Length

KW : Kernel Width

KT : Kernel Thickness

KWt : Kernel Weight

KNR : Kerbel Nut Ratio

DKR : Double Kernel Ratio

SoS : Softness of Shell

NS : Nut Shape

SCI : Shell Color Intensity

KCI : Kernel Color Intensity

SoK : Shriveling of Kernel

cv. : cultivar

PV : Phenotypic Variation

1. INTRODUCTION

Almond (*Prunus dulcis* (Mill)) D.A. Webb) along with other fruit crops such as peach, sweet cherry and apricot belong to the Prunoideae subfamily of Rosaceae. As same as many other *Prunus* species, it has the chromosome number of 2n=16 (Darlington, 1930). Almond is valued for its health benefits and high nutritional value, the importance of the crop is increasing in the human diet, and consequently, its production and commercial value are growing worldwide. However, it has been much less studied compared to other Rosaceous fruits.

Almond's domestication by humans can date back to the third millennium BC (Spiegel-Roy, 1986). Considered one of the oldest tree crops has been domesticated and has been grown in the Mediterranean Basin at least for the last 2,500 years (Grasselly and Crossa-Raynaud, 1980). Then almond had spread to the other parts of the world with carried seeds by caravans on the old Silk Road. Currently, almond is mainly grown in the Mediterranean Basin, the Middle East, south-western Asia, the USA, and Australia. The almond is currently cultivated in both the northern hemisphere and southern hemisphere and it is one of the most important nut crops in terms of world production.

In 2008, world cultivation area of almond was 1.48 million hectares, while in 2012 that number increased to 1.59 million hectares and in 2018 the number increased again to 2.06 million hectares. Production has increased from 1.5 million tons to 3.3 million metric tons from 2001 to 2018. In recent years, the USA with its 57% portion of the world's almond production ranks the first in the world almond production (Table 1; Faostat, 2020).

Table 1.1. Worlds almond production (metric tons).

Countries / Year	2014	2015	2016	2017	2018
USA	1,545,500	1,787,033	2,002,742	1,476,539	1,872,500
Spain	195,704	211,084	202,339	255,503	339,033
Iran	136,338	146,000	147,863	129,566	139,029
Morocco	101,026	97,723	112,681	116,923	117,270
Turkey	73,230	80,000	85,000	90,000	100,000
Italy	74,016	70,399	74,584	79,599	79,801
Australia	55,978	165,018	72,902	75,373	69,880
Tunisia	66,700	70,500	61,000	67,000	66,733
Syria	34,729	84,634	88,841	54,939	61,676
Algeria	64,827	76,482	66,095	61,934	57213
TOTAL	**2,635,528**	**3,066,032**	**3,214,303**	**2,760,166**	**3,259,756**

Turkey has a strong potential in almond production. Almond production has increased from 52 thousand metric tons to 100 thousand metric tons from 2008 to 2018. And that equals 3.1% of the whole world's almond production. In recent years, Turkey has surpassed Italy and Australia become the fifth-largest producer of almond in the world. (Table 1; Faostat, 2020).

With the increase in the commercial value of almond worldwide, many countries are dedicated to develop new cultivars. The main objective of these almond breeding programs is high yield. Two main factors affect the productivity of almond: (1) Blooming date: Being the earliest blooming species in temperate climate fruits, late-blooming cultivars are very important to escape the early spring frost damage which threatens the production (Socias i Company et al., 2012). Late-blooming cultivars are more important in inland regions where the orchards have more risk of frost damage. (2) Self-compatibility: Because of the self-incompatible nature of almond, insufficient cross-pollination is a major cause of low yield. Under the same environmental and breeding conditions, production of the self-compatible cultivars

per tree and per area is up to two times higher than the most productive self-incompatible cultivar (Godini et al., 2002).

Except for these two major objectives tree architecture, harvesting time and kernel quality are also important in almond breeding. Some major almond producing countries are constantly releasing new cultivars. For example, in the USA they release self-incompatible cultivar 'Sweetheart' which was fully compatible with 'Nonpareil' with high kernel quality (Gradziel et al., 2013).

Being one of the origins of almond, the Mediterranean, southeast Anatolia and Ege regions of Turkey are very suitable for almond cultivation. However, the production of Turkey is 20 times less than the USA (Faostat, 2020). In recent years, almond orchards have been established using mostly foreign cultivars in Turkey. However, all the local cultivars and most of the foreign cultivars are self-incompatible. Thus, it is urgent to start breeding programs in Turkey to release cultivars with late-blooming, self-compatible with high yield.

An almond cultivar breeding project that supported by TUBITAK (TOVAG 108 O 388 and TOVAG 113 O 963) was started in 2009 to breed self-compatible and late-blooming almond cultivars. In the first step, 4,082 F1 individuals were obtained from 12 different combinations. In the second step, marker-assisted selection (MAS) was applied to select self-compatible ones, later the breeding orchard was established with 1,870 self-compatible plants. The populations were also good genetic sources for genetic linkage mapping and QTL analysis studies, especially for marker development linked to economically important characters.

Plant breeding is a combination principle and method of changing the genetic constitution of a plant to make it more suitable for human needs. Conventional plant breeding has a very long history but in perennials like almond needs a long breeding period and requires lots of labor and expenses. Today, breeders combine conventional breeding and molecular breeding methods in plant breeding programs, Molecular breeding methods make plant breeding more efficient, produces high-quality varieties in a shorter period with low cost (Vinod, 2006).

Marker Assisted Selection (MAS) is a key step to improving the efficiency of the fruit-breeding programs. The best example of this is the self-compatible DNA marker that we used in the TUBİTAK project. If we didn't use the self-compatible marker to eliminate self-incompatible individuals, we had to deal with 4,082 progenies instead of 1,870. It was necessary to keep 2,212 self-incompatible progenies in the breeding orchard during a few years, and MAS in the TUBİTAK project provided lower cost, labor and land. Therefore, MAS is very important technique in almond breeding programs and we need more markers linked economically important traits in cultivar breeding programs.

Although MAS is very important for fruit species to improve the efficiency of the breeding program, but its usage was just limited to the traits with the low number of genes involved in the expression process (Salazar et al., 2014). The polygenic nature of the many important agronomical traits was distributed throughout the entire genome that makes it difficult to identify markers that closely linked to these traits. The construction of high-density linkage maps and quantitative trait loci (QTL) analysis are good strategies for identifying candidate genes associated with important traits. The high-density genetic linkage maps are needed to facilitate QTL mapping, compared genome analysis, and map-based cloning as well as predicting total breeding and genetic values for traits of agricultural significance (Paterson et al., 2009).

There are many types of molecular markers used for genetic map construction in almond such as isoenzyme, randomly amplified polymorphic DNA (RAPD), rRestriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) (Arus et al., 1994; Ballester et al.,2001; Dirlewanger et al., 2004; Gregory et al., 2005; Sanchez-Perez et al., 2007; Sanchez-Perez et al., 2010; Tavassolian et al., 2010; Fernandez i Marti et al., 2011). SSR is a very valuable marker system in genetic mapping studies due to its co-dominance nature, especially in outbreeding F1 populations. Because, maps of parents have to be constructed separately in F1 populations and co-dominant

markers such as SSRs are necessary to combine both genetic maps. This makes the SSRs very useful marker technique for constructing a genetic linkage map. However, SSRs are not abundant in the genome compare to single nucleotide polymorphisms (SNPs) markers, which appears very frequently in plant genomes. Additionally, development of SSR markers is very time consuming and needs a lot of lab work. Most of the previous QTL studies were not able to identify the accurate positions of the QTL and find markers that closely related to the important traits due to the low marker density in the linkage maps. So SSR markers being the marker of choice for mapping studies combined with other much more abundant markers such as SNPs to construct high-density linkage maps (Donoso et al., 2016).

With the advances in next-generation sequencing (NGS) technologies, new genotyping methods emerged to meet the demands for fast and cost-effective genotyping and sequencing in the plant genome. Such as restriction site-associated DNA sequencing (RAD-seq) and genotyping-by-sequencing (GBS) and DArT-seq which use NGS as a platform. DArT-seq represents a new implementation of sequence reduced representations. Because it is optimized for each organism by selecting the most appropriate complexity reduction method it gained popularity rapidly as a GBS method. DArT-seq have been applied successfully in many plant species (Killian et al., 2012; Cruz et al., 2013; Raman et al., 2014). In standard DArT assay, approximately 200,000 genomic fragments are sequenced 10 times, and most of the samples experiment in duplicate in order to make sure the reproducibility. After very strict filtering processes DArT-seq offers affordable genome profiling through the generation of high-density SNPs and silico DArT (presence/absence of restriction fragments in representation) markers.

There are many high-density linkage mapping and QTL analysis studies using these linkage maps in different *Prunus* species such as; sweet cherry (Klagges et al.,2013; Guajardo et al., 2015; Wang et al., 2015; Calle et al., 2018), peach (Eduard et al., 2013; Martinez-Garcia et al., 2013; Frett et al., 2014; Bielenberg et al., 2015; Silva Linge et al., 2018; Nunez-Lillo et al., 2019), Japanese plum (Salazar

et al., 2017), sour cherry (Cai et al., 2018) and Japanese apricot (Kitamura et al., 2018). All of the above studies constructed relatively high-density linkage maps and these linkage maps became very useful in detecting of QTL regions accurately. However, there is one linkage mapping study using SNP markers available in almond with only 309 SNP markers (Goonetilleke et al., 2018). Therefore, it is necessary to develop a high-density linkage map in almond.

Main objectives of this study are:

1. To contruct high-density linkage maps in 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' almond populations by using SSR, SNP and DArT markers.
2. Identify QTL regions related to nut and kernel traits of almond to find markers linked to these QTLs.

In this study, previously constructed SSR-based genetic linkage maps of 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' F1 populations (Paizila, 2016) were used as framework maps. These new well-saturated almond linkage maps present valuable sets of newly identified SNPs and silico DArT markers, provide a powerful tool for QTL identification and further breeding applications and provides almond genomic information for the international community.

2. LITERATURE REVIEWS

2.1. Genetic Linkage Map and QTL Studies in Almond

The first almond genetic map was constructed in 'Ferragnes x Tuono' F1 population (60 F1 individuals) by using RFLP and isozymes markers (Arus et al., 1994). Viruel et al. (1995) used 120 RFLP and 7 isozyme markers in the same population, Joobeur et al. (2000) added 54 RAPD and 6 SSR markers to the same map in order to construct the more saturated map. Ballester et al. (1998) mapped the S locus in linkage group 6 (LG6).

Ballester et al. (2001) constructed a genetic linkage map of almond in 'Felisia x Bertina' F1 population (134 F1 individuals). The authors mapped a total of 81 RFLP and 5 RAPD markers.

The most important map in *Prunus* species is the 'Texas x Earlygold' map mostly referred as T x E map, which widely accepted as a reference map for the genus. The latest version of this map included 562 markers (361 RFLPs, 185 SSRs, 11 isozymes and 5 STSs) covered a total distance of 519 cM and the average map density was 0.92 cM/marker and the largest gap was 7 cM with a late-blooming locus on LG4 (Dirlewanger et al., 2004).

Sanchez-Perez et al. (2007) constructed a genetic map of almond using 56 SSRs and 11 traits in 'R1000 x Desmayo Largueta' F1 population (167 F1 individuals), and did QTL (Quantitative Trait Loci) analysis of a few traits such as self-compatibility, blooming date, blooming density, ripening time, leafing date, productivity, kernel taste, in-shell weight, shell hardness, kernel weight, double kernel and mapped them on the linkage map.

Sanchez-Perez et al. (2010) mapped sweet kernel (Sk) locus in LG5 using the 'R1000 x Desmayo Largueta' F1 population. Then, the authors tested 15 more SSR primer pairs that were previously mapped in LG5 by Howard et al. (2005) and they found several SSR markers that are closely linked to the Sk locus.

Sanchez-Perez et al. (2012) identified QTLs related to the chilling and heat requirements, flowering time using the 'R1000 x Desmayo Largueta' F1 population. A major QTL for the chilling requirement was found in LG4. Two QTL regions related to the heating requirement was identified in LG2 and LG7. A major QTL for the flowering time was found in LG4.

Gregory et al. (2005) used RAPD, ISSR, SSR and morphological markers in 'Nonpareil x Lauranne' F1 population (93 individuals) to construct a parental genetic linkage map of almond. However, among the tested 120 markers the authors mapped only 12 markers with a total length of 161.9 cM.

Wu et al. (2009) mapped 12 SNP-anchored putative genes in the 'Nonpareil x Lauranne' population using HRM (High-Resolution Melting) analysis, 12 putative genes mapped in six linkage groups.

Rabiei et al. (2009) mapped the QTL related to the flowering time in the 'Nonpareil x Lauranne' population with two-year observation data and mapped the flowering time gene in LG6.

Tavassolian et al. (2010) tested 241 SSR primers pairs in the parents and F1 in the 'Nonpareil x Lauranne' population, and 108 were segregated, and 96 were mapped. The authors also mapped 35 ISSR, 14 SNP, 4 S-alleles and 11 RAPD markers in this population. However, the authors indicated that different populations planted in different ecologies are necessary in order to widely develop genetic markers, and map some of the genes in future studies.

Fernandez i Marti et al. (2011) used 77 F1 plants derived from 'Vivot x Blanquerna' population to find QTLs related to the self-compatibility. They tested 102 SSR loci from T x E ('Texas x Earlygold') reference map, and 52 markers were mapped. The authors detected two QTL regions in LG6 and LG8 related to the self-compatibility.

Forcada et al. (2012) used the 'Vivot x Blanquerna' population for QTL analysis of the nut chemical contents and tested 8 more SSR markers and added 4 more SSR markers into the map. That makes the total SSR loci number mapped into

‘Vivot x Blanquerna’ population to 56 and also mapped QTLs related to fat, fatty acids, tocopherol, and protein contents.

Fernandez i Marti et al. (2013) used the ‘Vivot x Blanquerna’ population and same linkage map to mapped 14 putative quantitative trait loci of the physical nut trait into the six genomic regions of the eight almond linkage groups.

Donoso et al. (2016) studied 42 traits for flowering, phenology, fruit quality, leaf and disease resistance in ‘Texas x Earlygold’ population and its BC1 and T1E population. For genetic mapping, they used 135 SSR markers and SNP markers from the 9k international peach SNP consortium. As a result, they mapped a major QTL for pistil length in LG6. For shooting and blooming time, they detected six major QTLs in LG1, LG6, LG7, LG8 and two in LG2. A large QTL for juiciness was found in T x E map at the end of LG1.

Goonetilleke et al. (2018) 309 SNP primer pairs (146 designed were heterozygous in Nonpareail and 162 designed were heterozygous in Lauranne) were used for mapping in 231 F1 progenies derived from a cross between 'Nonpareil x Lauranne'. Shell hardness data was evaluated for a random 10 samples for each sample. A total of 180 F1were used for QTL detection. As a result, two QTLs for shell hardness were detected on LG5 of Nonpareil map. However, in Lauranne map the same QTLs were detected on LG2, LG5, and LG8.

Sanchez-Perz et al. (2019) sequenced the whole genome of almond cv. Lauranne. They also revealed the mechanism of bitterness in almond, which leads to the domestication of toxic wild almond species. They detected a point mutation in the 46-kilobase gene cluster that encodes five basic helix-loop-helix transcription factors. Among them, bHLH2 controls the P450 monooxygenase-encoding genes involved in the biosynthetic pathway of amygdalin. They also pointed out that a nonsynonymous point mutation in the dimerization domain of bHLH2 prevents transcription of two cytochrome p450 genes resulting in the sweet kernel trait in almond.

Alioto et al. (2020) sequenced the whole genome of almond cv. Texas. They obtained a total of size of 227.6Mb sequence, among them 91% of the sequence anchored on the eight pseudomolecules of almond. They also compared the genome of almond to other 16 other species and find out that almond and peach diverged around 5.88 million years ago. They also detected many presence/absence variants between two species, which may lead to the divergence of two species. They believe that transposable elements (TE) play an important role in the phenotypic difference between the two species. For example, sweet kernel trait in almond is correlated with the reduced expression of the highly methylated TE insertion which involved in amygdalin biosynthesis.

2.2. Genetic linkage map and QTL studies in other *Prunus* species

Klagges et al. (2013) tested 5,696 SNP markers in two intra-specific sweet cherry progenies. 89 F1 individuals from 'Black Tartarian x Kordia' and 121 F1 individuals from 'Regina x Lapins' populations. As a result, a total of 723 and 687 markers were mapped with a total length of 752.9 and 639.9 cM in the 'BT x K' and 'R x L' population respectively. The average markers distance of the 'BT x K' and 'R x L' populations were 1.1 and 0.9 cM, respectively.

Guajardo et al. (2015) constructed linkage maps in intra-specific sweet cherry cross 'Rainier x Rivedel' using SSR and SNP markers that developed from GBS studies. A total of 462,489 and 985 markers were mapped in Rainier, Rivedel and consensus map, respectively. The average distance of markers in parental maps was 1.2 cM. While in consensus map the average distance of the markers was 0.7 cM.

Wang et al. (2015) mapped 701 SNP markers developed from specific-locus amplified fragment (SLAF) method, 16 SSR markers and the incompatibility gene to the sweet cherry 'Wanhongzhu x Lapins' population linkage map and try to detect QTLs related to the trunk diameter. All the markers spanned on 8 LGs with a total map length of 849.0 cM. The marker density was 1.18 cM and they mapped 4 loci related to trunk diameter in three different linkage groups.

Calle et al. (2018) constructed two genetic linkage maps in one self-pollinated F2 sweet cherry population that derived from Cristobalina, one interspecific crossed F1 population of 'Vic x Cristobalina'. 511 and 816 SNP markers were mapped on F2 and F1 population covering 622.4 and 726.0 cM, respectively. Then they compared the mapped SNP marker position orders to the sweet cherry and peach reference genome and observed a high level of synteny between the linkage map and reference genomes. However, they find a noticeable inversion on the top of LG5. And they detected many homozygous regions in the self-pollinated F2 map compared to the crossed F1 map.

Eduardo et al. (2013) try to map volatile organic compounds (VOCs) in a peach population created from 'Bolero x OroA' with differing aroma profile. The population has 126 seedlings and VOCs values were collected for two years. First, the constructed a linkage map using SNP and SSR markers. As a result, 231, 87 and 123 markers were mapped in Bolero, OroA and 'Bolero x OroA' maps, respectively. All of the maps do not consist of 8 LGs. However, they identified a total of 72 QTLs for studied 23 VOCs.

Martinez-Garcia et al. (2013) constructed a high-density SNP linkage map in two peach population and studied QTL related to fruit quality characters in peach.69 F1 seedling from the cross between Dr. Davis and F8, 1-42 (DF) and 55 F1 progenies from the cross between 'Dr. Davis x Georgia Belle' (DG) were phenotyped for mealiness (M), flesh bleeding (FBL) and flesh browning (FBr) for three years. A total of 1,400 SNP markers were developed, among them 738 and 588 markers were finally mapped on DF and DG populations, respectively. The linkage maps were compared with the peach physical map and showed several differences with the physical map. Significant QTLs related to the M and FBL were detected on LG4 and QTL for FBr was identified on LG5.

Frett et al. (2014) studied QTL related to blush in peach. They used 25 F2 progeny derived from the self-cross of the F1 individuals of a Zin Dai and Crimson Lady (ZC^2) cross. Four years of blush phenotypic data were collected. A total of

1,335 SNP markers were successfully mapped in ZC^2 map. A total of four QTLs were identified related to the blush in peach. Of which, one major QTL on LG3 and there minor QTLs on LG4 and LG7. Skin coloration related candidate genes were located on the major QTL region on LG3. Silva Linge et al., (2018) analyzed 65 more F2 individuals in ZC^2 population and constructed a linkage map using a total of 90 progenies. They also mapped 1,476 SNP markers on the linkage map. The consensus map of the ZC^2 included a total of 2,975 SNP markers, 116 SSR markers, and one morphological marker. This consensus map will valuable for QTL detection studies in *Prunus* species.

Bielenberg et al. (2015) used the GBS platform to develope SNP marker in the peach F2 population which is the self cross of the F1 progenies of the cross between Hakuho and UFGold. Blooming date (BD) and chilling requirement (CR) were phenotyped in 57 F2 progenies for seven years and two years, respectively. 201 SNP and 33 SSR markers were mapped on the F2 map over 8 LGs with a total map distance of 666.1 cM. For CR eight QTLs were detected in the first year and two QTLs detected in the second year. For BD 19 QTLs were identified for seven years.

Nunez-Lillo et al. (2019) studied soluble solid content (SSC), mealiness (M) and maturity date (MD) in an intraspecific cross between peach OHenry and nectarine NR-053. A total of 194 F1 progeny derived from this cross-population and all the phenotypic data were collected for three years. At first step, they constructed a linkage map with 499 markers which mostly SNP markers spanned over 8 LGs with a genetic length of 717.6 cM. Then the successfully detected QTLs for SSC and M on LG5 and for MD in LG1, LG2, LG5 and LG6. Finally, they also look for the genes related to these traits. For SSC they found four transcription factors and one gene, one cell wall gene identified for M and more than 20 genes were detected to be related to MD.

Salazar et al. (2017) mapped a total of 981 SNP markers developed by GBS in '98-99 x Angeleno' Japanese plum population. 153 F1 individuals were used for linkage map construction. Among them, 55 seedlings were phenotyped for fruit

quality and repining time in 2015 and 2016. And conducted QTL analysis and successfully mapped repining time in LG4 and fruit skin color in LG3 and LG4 in both parents in both years. They also mapped the chlorophyll degradation index in LG3 and fruit weight in LG7 in both parents in one of the years.

Zeballos et al. (2016) constructed a linkage map in nectarine population 'Venus x Big Top' using 411 SSR and SNP markers. 104 markers were mapped in Venus linkage map spanned over 9 LGs with a total map length of 259 cM. While the Big Top map consists of 122 markers with 10 LGs. A total of 54 QTLs were detected over 12 LGs. LG4 of the Venus and LG5 of the Big Top has the highest number of QTLs. And many of the QTLs such as; total phenolic, anthocyanin and flavonoids were reported the first time.

Cai et al. (2018) total of 338 sour cherry F1 individuals derived from five sour cherry populations was used to construct genetic linkage map using 5696 SNP makers. All the individuals were also phenotyped for blooming date in three years. As a result, a total of 1795,1795,1807,1787 and 1856 SNP markers were mapped from Pop1 to Pop5, respectively. Four QTLs related to the blooming date were detected on LG1, LG2, LG4, and LG5.

Kitamura et al. (2018) studied blooming date (BD), leafing date (LD), chilling requirement (CR) and heat requirement (HR) in Japanese apricot. They constructed high-density linkage maps in 101 progenies from 'Nanko x SC' (NKSC) population and 63 progenies from the 'Ellching x Nanko' (NINK) used SNP markers that developed by GBS. A total of 408 and 718 markers were mapped on NKSC and NINK maps, respectively. The average markers distance in NKSC was 1.62 cM compared to the 1.83 cM in NINK. This study detected significant QTLs related to CR, LD, and HR on LG 4 on both populations.

3. MATERIAL AND METHODS

3.1. Materials

In this study, two F1 populations from a TÜBİTAK project (TOVAG 108 O 388) were used. In the first population, local cultivar Gülcan-2 and French cultivar Lauranne were used as maternal and paternal parents, respectively. In the second population, Spanish cultivar Guara and local cultivar Nurlu were used as maternal and paternal parents, respectively. The cultivars Lauranne and Guara are self-compatible, while Turkish local cultivars Gülcan-2 and Nurlu are self-incompatible. A total of 107 and 114 F1 progenies were used in 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations, respectively. The F1 plants were planted during the winter in 2011-2012 in Pistachio Research Institute in Gaziantep province of Turkey.

Gülcan-2: This cultivar is a selection from the nature in a selection breeding program in the Ege region. It is a late-blooming and self-incompatible cultivar. Nut weight is around 3 g; kernel weight is around 0.8 g. It has high yield production and late ripening. The percentage of the double kernel is around %10 (Açar, 2012).

Lauranne: It is a cultivar released from French's INRA Institute, derived from the cross of 'Ferragnes × Tuono'. Late-blooming and self-compatible cultivar. It has an intermediate hard shell. Kernel percentage is 29-35% and the double kernel percentage is 5-20 % (Açar, 2012).

Guara: Released by CITA Institute of Spain, is a widely cultivated cultivar in Spain. Late-blooming and self-compatible cultivar. Kernel percentage is 30-34% and the double kernel percentage is 10-20% (Açar, 2012).

Nurlu: It is a good cultivar found in Datça and has an extremely hard shell. Kernel percentage is 31% without double kernel. It has a good taste and the large kernel is a highly attractive cultivar in the markets (Açar, 2012).

3.2. Methods

3.2.1. Phenotypic data collection

All the phenotypic data of two almond populations 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' were collected during two consecutive years (2017 and 2018) according to the almond descriptors by Gülcan (1985). A total of 21 characters related to nut and kernel quality were studied. Additional two years data obtained by Ercik (2018) for the same characters were also used in QTL analysis.

3.2.1.1. Nut and kernel color

HunterLab instrument was used for measuring of both nut and kernel color in five replicates for each progeny.

3.2.1.2. Softness of shell

There are 5 parameters for shell softness as shown below:

Table 3.1. Softness of almond shell.

Parameter	Score	Reference
Extremely hard	1	Bartre
Hard	3	Desmaye Largueta
Intermediate	5	Texas
Soft	7	Princesse
Paper	9	Nonpareil

3.2.1.3. Nut shape

Nut shape was scored on a scale of 1-5 according to Figure 1.

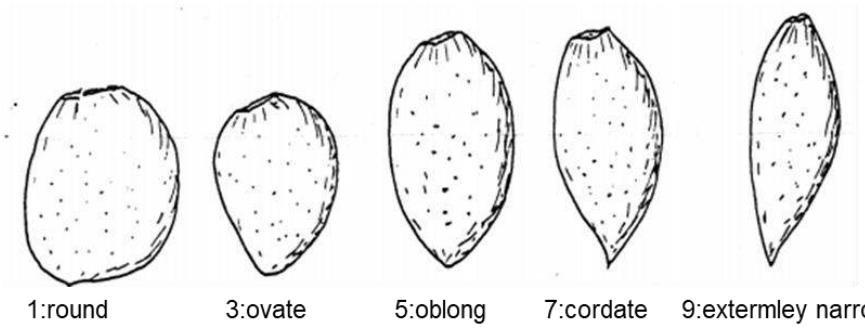

Figure 3.1. Nut shape of an almond.

3.2.1.4. Shriveling of kernel

Shriveling of the kernel was scored as slightly wrinkled (1), intermediate (5) and wrinkled (7).

3.2.1.5. Nut and kernel sizes (length, width and thickness)

Nut and kernel length, width and thickness were measured using a digital caliper (mm) in randomly selected 30 nuts from each progeny.

3.2.1.6. Nut and kernel weight (g)

Both nut and kernel were weighted using a digital scale with 0.01 g sensevitiy. A total of randomly selected 30 nuts were used for nut and kernel weight.

3.2.1.7. Kernel percentage (%)

Kernel percentage is the percentage of the kernel weight to the nut weight%.

Kernel weight percentage% =kernel weight/ nut weight x100

3.2.1.8. Percentage of double kernels

The percentage of the double kernel was calculated according to the number of double kernels within randomly selected 30 nuts from each progeny.

3.2.1.9. Shell and Kernel color intensity

In order to score the color intensity of the shell and kernel (endocarp). All the genotypes were scored with 5 parameters which are extremely light, light, intermediate, dark, and extremely dark. 5 parameters will be scored according to Table 4.

Table 3.2. Shell and kernel color scores.

Shell and kernel color intensity	Score
Extremely light	1
Light	3
Intermediate	5
Dark	7
Extremely dark	9

3.2.2. DNA extraction

DNA was extracted from young leaf tissues by CTAB method (Doyle and Doyle, 1987) with some modifications (Kafkas et al., 2005). 80 mg of lyophilized leaves were ground with Tissue Lyser (Qiagen). A total of 0.9 ml of CTAB (100 mMTris-HCl, 1.4 M NaCl, 20mM EDTA, 2% CTAB, 2% PVP, 0.1% Na2S2O5) was added to the 2 mL tube that contains grounded leaves. Then, the samples were incubated in a water bath for an hour at temperature 65^0C. After 1 hour, samples were taken and were kept at room temperature for 5-15 minutes. A total of 900 µl of chloroform: isoamyl alcohol (24: 1) mixture was added to each tube for 15 minutes at room temperature. All samples were centrifuged at 14,000 rpm for 15 minutes. The aqueous phase (top layer) was transferred into the new tube (1.5 ml) and 700 µl of cold isopropanol (stored at the fridge) was added. Then, the tubes were mixed by inverting tubes very gently. The samples were left at -20^0C for overnight to precipitate the DNA, and the supernatant was discarded. Then, the DNA was washed with 10 mM ammonium acetate in 76% ethanol. The supernatant was again removed,

and the pellet was dried at room temperature. The dried DNA was diluted with TE buffer (10 mM Tris: 0.1 mM EDTA; pH 8.0).

3.2.3. Quantification of DNA

DNA concentrations were estimated on 0.8% agarose gel. 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 UV light source that causes DNA/dye to become visible. The DNA concentration was estimated by comparing λ DNA with known concentrations (25ng- 50ng- 100ng- 200ng). All the DNA samples were diluted to the concentration of approximately 80 ng for DArT-seq analysis.

3.2.4. High-throughput genotyping using DArT-Seq method

All the DNA samples were sent to DArT PL, Canberra, Australia for analysis. In DArT-seq technology restriction enzymes PstI and MseI were selected as restriction enzyme combination for genom complexity reduction. DNA samples were processed in digestion/ligation reactions. Then replacing the *Pst*I-compatible adapter with two different adapters corresponding to two different restriction enzyme overhangs. The *Pst*I-compatible adapter was designed to include Illumina flowcell attachment sequence, sequencing primer sequence and varying barcode region similar to the sequence reported by Elshire et al. (2011).

In standard DArT assay, approximately 200,000 genomic fragments are sequenced 10 times, and most of the samples experiment in duplicate in order to make sure the reproducibility. After very strict filtering processes DArT-seq offers affordable genome profiling through the generation of high-density SNPs and silico DArT (presence/absence of restriction fragments in representation) markers.

3.2.5. Construction of the linkage maps

Linkage maps were constructed for each parent independently using the double pseudo-testcross mapping strategy (Grattapaglia and Sederoff, 1994). The linkage analysis was performed using JoinMap version 4.1 (Van Ooijen, 2013). Segregation data were coded as indicated in the software. The segregating markers were tested for goodness of fit to hypothesized Mendelian ratios by using the chi-square (X^2) test with each locus analysis module of JoinMap 4.1 (Van Ooijen, 2013). The log of odds (LOD) scores based on G2 statistics was calculated using Joinmap for the recombination frequency to determine linkage groups. In the mapping process using JoinMap, a few markers were excluded from the final maps due to the difficulty to determine their linkage phases. Markers were also removed when their distorted segregation conflicted with the segregation pattern of neighboring markers.

For all maps, a LOD score of 5.0 was initially sets as the linkage threshold to determine markers in the same linkage group, and the standard JoinMap parameter settings were applied. In order to convert recombination frequencies into map linkage distance in centiMorgans (cM), Kosambi's mapping function (Kosambi, 1994) was used. The regression mapping algorithm was used for map construction. The presentation of the maps was done by using MapChart 2.2 for Windows (Voorrips, 2002).

3.2.6. QTL analysis

MapQTL v5.0 (Van Ooijen, 2008) was used for the analysis of QTL data using an interval mapping (IM) and multiple QTL model (MQM) approach; detection of QTLs was done by genetic linkage maps for each parent separately. Firstly, putative QTLs with LOD scores greater than 2.0 in datasets were reported using IM mapping approach. Then, the closest marker to the LOD peak of each significant QTL in interval map (IM) was used as a cofactor. Then, multiple QTL model mapping (MQM) was performed. The LOD significant thresholds for the genome-wide level was determined by running 1,000 permutation test. The

significance threshold *P* value 0.05 was used to detect significant QTLs. Furthermore, the Kruskal-Wallis test (KW test) was performed to verify the identified QTLs. A total of four years data was used in QTL analysis. Two years (2017 and 2018) data were collected in this study and another two years data (2015 and 2016) were previously obtained by Ercik (2018).

4. RESULTS AND DISCUSSION

In this study, we aimed to construct high-density linkage maps using 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations. We used the previously constructed SSR-based genetic linkage maps of 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations (Paizila, 2016). These framework linkage maps were enriched with a large number of SNPs and DArT markers in this study. Two years (2017 and 2018) of phenotypic data related to nut and kernel characteristics such as Shell L (SL), Shell a (Sa), Shell b (Sb), Kernel L (KL), Kernel a (Ka), Kernel b (Kb), Nut Length (NLh), Nut Width (NW), Nut Thickness (NT), Nut Weight (NWt), Kernel Length (KLh), Kernel Width (KW), Kernel Thickness (KT), Kernel Weight (KWt), Kernel Nut Ratio (KNR), Double Kernel Ratio (DKR), Softness of Shell (SoS), Nut Shape (NS), Shell Color Intensity (SCI), Kernel Color Intensity (KCI) and Shriveling of Kernel (SoK) were collected. In QTL analysis, phenotypic data from four years were used in which two years of data (2015 and 2016) were from the PhD thesis of Ercik (2019).

4.1. Construction of high density genetic linkage maps in almond

In this study, we constructed high-density parental linkage maps of Gülcan-2, Lauranne, Guara and Nurlu cultivars using SSR, DArT and SNP markers, and detailed information about each linkage group from each parental map were presented in Table 4.1, Table 4.2, Table 4.3 and Table 4.4, respectively. Due to the high number of markers in each linkage group, marker names cannot be visualized on the linkage groups. Therefore, names of the mapped markers, positions of the markers, segregation types of the markers and significance values of all markers in Gülcan-2, Lauranne, Guara, and Nurlu linkage maps were given in Supplementary file 1, Supplementary file 2, Supplementary file 3, Supplementary file 4, respectively.

4.1.1. Genetic linkage maps of ‘Gülcan-2 x Lauranne’ F1 population

A total of 107 F1 individuals of the ‘Gülcan-2 x Lauranne’ population were used for linkage map construction using DArT, SNP and SSR markers. All the DArT, SNP and SSR distributed along with the 8 haploid chromosomes of almond. A total of 3,338 (3,222 DArT and SNP, and 116 SSRs) markers mapped in the 'Gülcan-2' linkage map, while Lauranne paternal map included a total of 3,442 markers (3,318 DArT and SNP, and 124 SSRs). The length of each linkage group, the number of the mapped markers, average distance between markers, marker density, number and rate of the distorted markers were presented in Table 4.1 and Table 4.2. Each linkage group in ‘Gülcan-2 x Lauranne’ F1 population was explained in detail below.

Table 4.1. Length of each linkage group, the number of the mapped markers, average distance between markers, marker density, number and ratio of the distorted DArT, SNP and SSR markers in 'Gülcan-2' linkage map.

Gülcan-2												
LG	LG	The number of mapped markers				Average distance between markers	Marker density	The number of distorted markers				% Distortion
	Length	Dart	SNP	SSR	Total		(marker/cM)	Dart	SNP	SSR	Total	
1	68.73	213	106	18	337	0.20	4.90	73	16	12	101	29.97
2	64.17	398	191	22	611	0.11	9.52	70	28	11	109	17.84
3	66.24	336	160	17	513	0.13	7.74	17	0	7	24	4.68
4	56.32	264	130	14	408	0.14	7.24	87	10	7	104	25.49
5	47.89	216	105	12	333	0.14	6.95	62	4	1	67	20.12
6	52.23	308	165	7	480	0.11	9.19	39	3	0	42	8.75
7	51.57	115	96	14	225	0.23	4.36	89	25	12	126	56.00
8	62.71	275	144	12	431	0.15	6.87	107	30	4	141	32.71
Total	469.86	2,125	1,097	116	3,338			544	116	54	714	
Mean	58.73	265.63	137.13	14.50	417.25	0.15	7.10	68.00	14.50	6.75	89.25	24.45

Table 4.2. Length of each linkage groups, the number of the mapped markers, average distance between markers, marker density, number and rate of the distorted DArT, SNP and SSR markers in Lauranne linkage map.

Lauranne												
LG	LG	The number of mapped markers				The average distance of markers	Marker density	The number of distorted markers				% Distortion
	Length	Dart	SNP	SSR	Total		(marker/cM)	Dart	SNP	SSR	Total	
1	98.60	214	132	21	367	0.27	3.72	11	8	11	30	8.17
2	68.60	472	189	25	686	0.10	10.00	91	13	11	115	16.76
3	73.33	238	146	17	401	0.18	5.47	71	11	9	91	22.69
4	72.67	193	148	12	353	0.21	4.86	118	33	7	158	44.76
5	76.97	216	99	14	329	0.23	4.27	0	0	0	0	0.00
6	59.09	287	180	9	476	0.12	8.06	19	0	0	19	3.99
7	61.46	185	135	14	334	0.18	5.43	45	10	9	64	19.16
8	61.56	323	161	12	496	0.12	8.06	35	1	3	39	7.86
Total	572.27	2128	1190	124	3442			390	76	50	516	
Mean	71.534	266	148.75	15.5	430.25	0.18	6.23	48.75	9.5	6.25	64.5	15.43

4.1.1.1. LG1

LG1 of Gülcan-2 map was constructed by 337 (213 DArT, 106 SNP and 18 SSR) markers with a total map length of 68.73 cM. The average distance between markers was 0.20 cM and the average marker density was 4.90 markers per cM. LG1 of Lauranne cultivar included 214 DArT, 132 SNP and 21 SSR markers, and the total length was 98.60 cM. The average distance between markers was 0.27 cM and the average marker density was 3.72. The number of skewed markers in Gülcan-2 map was 101 (29.97%), while Lauranne map had 30 (8.17%) skewed markers (Figure 4.1, Table 4.1 and Table 4.2).

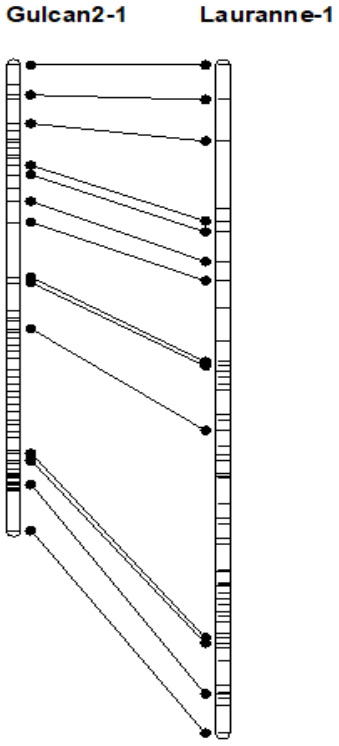

Figure 4.1. LG1 parental maps of ‘Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.1.2. LG2

LG2 in Gülcan-2 map consists a total of 611 markers. Of them, 398 were DArT, 191 were SNP and 22 were SSR markers. The LG2 in Gülcan-2 map had a total map length of 64.17 cM. The average distance between the markers was 0.11 cM and the average marker density was 9.52 markers per cM. LG2 in the Lauranne map included 686 (214 DArT, 132 SNP and 21 SSR) markers with a total length of 68.60 cM. The average distance between markers was 0.10 cM and the average marker density was 10.0 marker/cM. In LG2 of Gülcan-2 map, 17.84% (70 DArT, 28 SNP and 11 SSR) of the markers were skewed markers, while LG2 of Lauranne map had a total of 115 (16.76%) (91 DArT, 13 SNP and 11 SSR) skewed markers (Figure 4.2, Table 4.1 and Table 4.2).

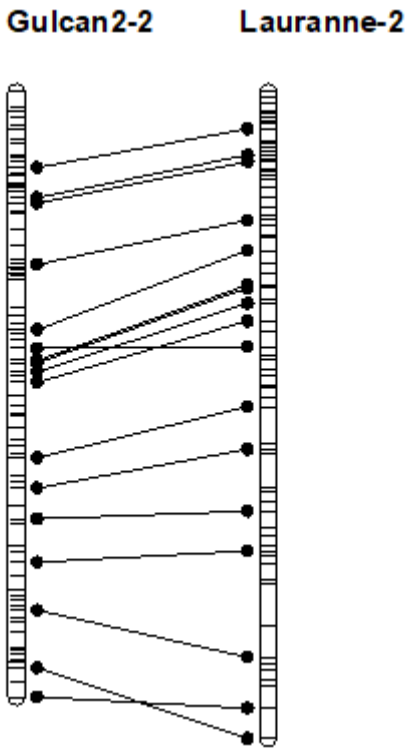

Figure 4.2. LG2 parental maps of ‘Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.1.3. LG3

LG3 of Gülcan-2 map was constructed by 513 (336 DArT, 160 SNP and 17 SSR) markers with a total map length of 66.24 cM. The average distance between the markers was 0.13 cM and the average marker density was 7.74 markers per cM. LG3 of Lauranne cultivar included 238 DArT, 146 SNP and 17 SSR markers, and the total map length was 73.63 cM. The average distance between markers was 0.18 cM and the average marker density was 5.47. The number of skewed markers in LG3 of Gülcan-2 cultivar was 24 (4.68%), while LG3 of Lauranne map had 91 (22.69%) skewed markers (Figure 4.3, Table 4.1 and Table 4.2).

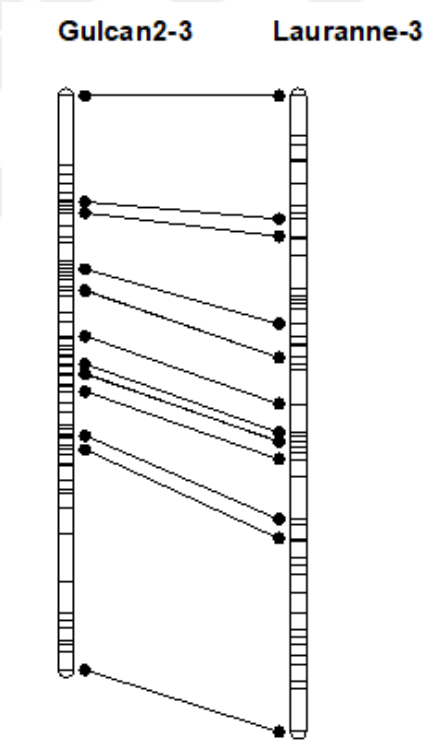

Figure 4.3. LG3 parental maps of ‘Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.1.4. LG4

LG4 of Gülcan-2 map consists a total of 408 markers. Of them, 264 were DArT, 130 were SNP and 14 were SSR markers. LG4 map of Gülcan-2 had a total map length of 56.32 cM. The average distance between the markers was 0.14 cM and the average marker density was 7.24 markers per cM. LG4 of the Lauranne cultivar included 353 (193 DArT, 148 SNP and 12 SSR) markers with a total length of 72.67 cM. The average distance between markers was 0.21 cM and the average marker density was 4.68. In LG4 of Gülcan-2 map, 25.49% (87 DArT, 10 SNP and 7 SSR) of the markers were skewed markers. While a total of 158 (44.76%; 118 DArT, 33 SNP and 7 SSR) markers showed skewed segregation in LG4 of Lauranne map (Figure 4.4, Table 4.1 and Table 4.2).

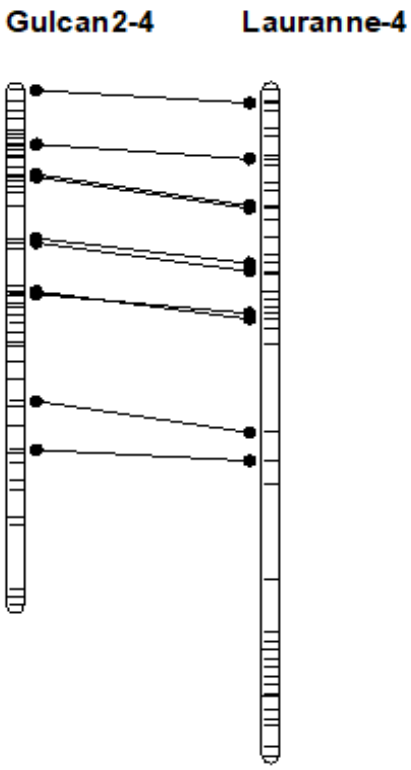

Figure 4.4. LG4 parental maps of ‘Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.1.5. LG5

LG5 of Gülcan-2 map was constructed by 333 (216 DArT, 105 SNP and 12 SSR) markers with a total map length of 47.89 cM. The average distance between the markers was 0.14 cM and the average marker density was 6.95 markers per cM. LG5 of Lauranne cultivar included 216 DArT, 99 SNP and 14 SSR markers, and the total map length was 76.97 cM. The average distance between markers was 0.23 cM and the average marker density was 4.27. The number of skewed markers in LG5 of Gülcan-2 cultivar was 67 (20.12%), while LG5 of Lauranne map had no skewed marker (Figure 4.5, Table 4.1 and Table 4.2).

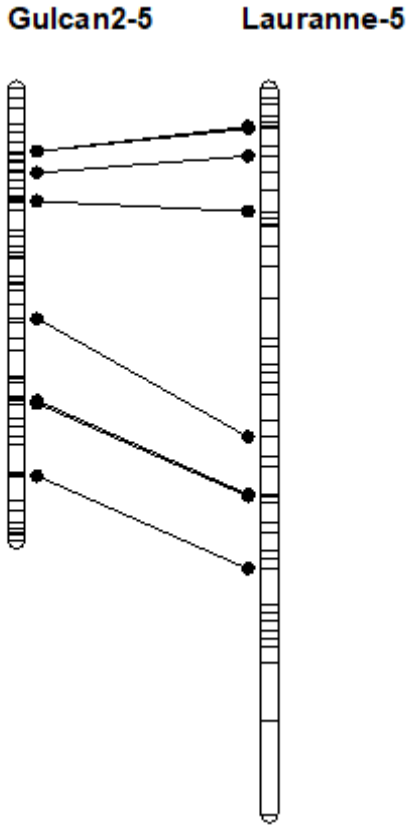

Figure 4.5. LG5 parental maps of ‘Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.1.6. LG6

LG6 of Gülcan-2 map consists a total of 480 markers. Among them, 308 were DArT, 165 were SNP and 7 were SSR markers. LG5 map of Gülcan-2 had a total map length of 52.23 cM. The average distance between the markers was 0.11 cM and the average marker density was 9.19 markers per cM. LG6 of the Lauranne cultivar included 476 (287 DArT, 180 SNP and 9 SSR) markers with a total length of 59.09 cM. The average distance between markers was 0.12 cM and the average marker density was 8.06. In LG6 of Gülcan-2 map, 8.75% (39 DArT, 3 SNP) of the markers were skewed markers, while 19 DArT (3.99%) markers showed skewed segregation in LG6 of Lauranne map (Figure 4.6, Table 4.1 and Table 4.2).

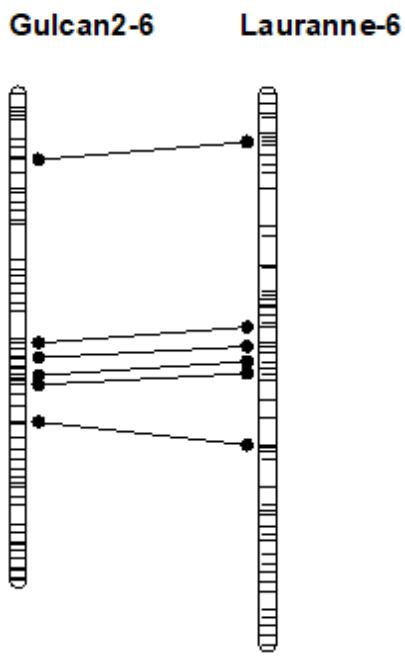

Figure 4.6. LG6 parental maps of 'Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.1.7. LG7

LG7 of Gülcan-2 map was constructed by 225 (115 DArT, 96 SNP and 14 SSR) markers with a total map length of 51.57 cM. The average distance between the markers was 0.23 cM and the average marker density was 4.36 markers per cM. LG7 of Lauranne cultivar included 185 DArT, 135 SNP and 14 SSR markers, and the total map length was 61.46 cM. The average distance between markers was 0.18 cM and the average marker density was 5.43. The number of skewed markers in LG7 of Gülcan-2 cultivar was 126 (56.0%), while LG7 of Lauranne map had 64 (19.16%) skewed markers (Figure 4.7, Table 4.1 and Table 4.2).

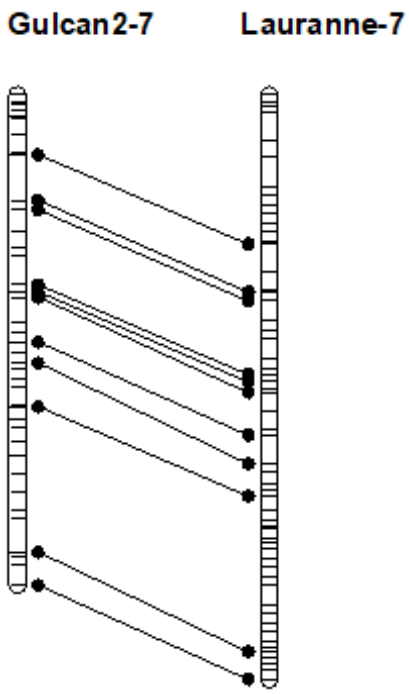

Figure 4.7. LG7 parental maps of ‘Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.1.8. LG8

LG8 of Gülcan-2 map consists a total of 431 markers. Among them 275 were DArT, 144 were SNP and 12 were SSR markers. LG8 map of Gülcan-2 had a total map length of 62.71 cM. The average distance between the markers was 0.15 cM and the average marker density was 6.87 markers per cM. LG8 of the Lauranne cultivar included 496 (323 DArT, 161 SNP and 12 SSR) markers with a total map length of 61.56 cM. The average distance between markers was 0.12 cM and the average marker density was 8.06. In LG8 of Gülcan-2 map, 32.71% (107 DArT, 30 SNP and 4 SSR) of the markers were skewed markers, while 35 DArT, 1 SNP and 3 SSR (7.86%) markers showed skewed segregation in LG8 of Lauranne map (Figure 4.8, Table 4.1 and Table 4.2).

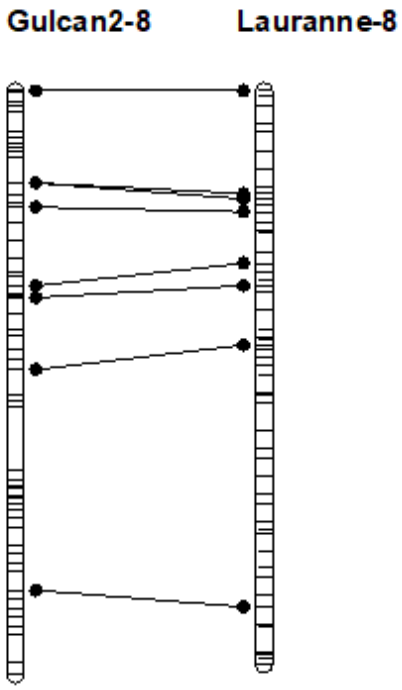

Figure 4.8. LG8 parental maps of ‘Gülcan-2 x Lauranne' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2. Construction of genetic linkage maps in 'Guara x Nurlu' population

A total of 114 F1 individuals of the 'Gülcan-2 x Lauranne' population were used for linkage map construction by using DArT, SNP and SSR markers.

In 'Guara x Nurlu' population, genetic maps of Guara and Nurlu parental cultivars were constructed. All the DArT, SNP and SSR markers distributed along with the 8 haploid chromosomes of almond. A total of 2,563 (1,210 DArT, 1,235 SNP and 118 SSR) markers were mapped in the Guara genetic linkage map, while a total of 3,135 markers (1,334 DArT, 1,669 SNP and 122 SSR) were mapped in Nurlu genetic linkage map. The length of each linkage group, number of the mapped markers, average distance between markers, marker density, number and rates of the distorted markers were presented in Table 4.3 and Table 4.4.

Table 4. 3. Length of each linkage group, the number of the mapped markers, average distance between markers, marker density, number and rates of the distorted DArT, SNP and SSR markers in Guara linkage map.

Guara												
LG	LG	The number of mapped markers				The average distance of markers	Marker density	The number of distorted markers				% Distortion
	Length	Dart	SNP	SSR	Total		(marker/cM)	Dart	SNP	SSR	Total	
1	73.13	194	211	19	424	0.17	5.80	13	1	1	15	3.54
2	52.87	254	225	22	501	0.11	9.48	13	8	8	29	5.79
3	62.16	163	192	17	372	0.17	5.98	59	16	6	81	21.77
4	51.71	126	152	13	291	0.18	5.63	57	28	1	86	29.55
5	54.15	124	115	11	250	0.22	4.62	7	0	0	7	2.80
6	52.72	27	28	15	70	0.75	1.33	19	6	13	38	54.29
7	47.76	143	161	10	314	0.15	6.57	5	0	1	6	1.91
8	49.43	179	151	11	341	0.14	6.90	97	23	1	121	35.48
Total	443.92	1,210	1,235	118	2,563			270	82	31	383	
Mean	55.49	151.25	154.38	14.75	320.38	0.24	5.79	33.75	10.25	3.88	47.88	19.39

Table 4.4. Length of each linkage group, the number of the mapped markers, average distance between markers, marker density, number and rates of the distorted DArT, SNP and SSR markers in Nurlu linkage map.

Nurlu												
LG	LG	The number of mapped markers				The average distance of markers	Marker density	The number of distorted markers				% Distortion
	Length	Dart	SNP	SSR	Total		(marker/cM)	Dart	SNP	SSR	Total	
1	108.54	233	359	18	610	0.18	5.62	55	38	1	94	15.41
2	57.24	215	172	20	407	0.14	7.11	48	13	6	67	16.46
3	55.96	117	139	14	270	0.21	4.82	5	1	4	10	3.70
4	56.33	156	201	12	369	0.15	6.55	1	0	1	2	0.54
5	57.55	96	139	12	247	0.23	4.29	1	0	0	1	0.40
6	57.53	182	301	17	500	0.12	8.69	0	0	10	10	2.00
7	64.33	133	170	15	318	0.20	4.94	1	0	1	2	0.63
8	58.55	212	188	14	414	0.14	7.07	2	0	1	3	0.72
Total	516.02	1344	1669	122	3135			113	52	24	189	
Mean	64.50	168	208.63	15.25	391.88	0.17	6.14	14.13	6.50	3.00	23.63	4.98

4.1.2.1. LG1

LG1 of Guara was constructed by 424 (194 DArT, 211 SNP and 19 SSR) markers with a total map length of 73.13 cM. The average distance between the markers was 0.17 cM and the average marker density was 5.80 markers per cM. LG1 of Nurlu cultivar included 233 DArT, 359 SNP and 18 SSR markers, and the total map length was 108.54 cM. The average distance between markers was 0.18 cM and the average marker density was 5.62. The number of skewed markers in LG1 of Guara cultivar was 15 (3.54%), while LG1 of Nurlu map had 94 (15.41%) skewed markers (Figure 4.9, Table 4.3 and Table 4.4).

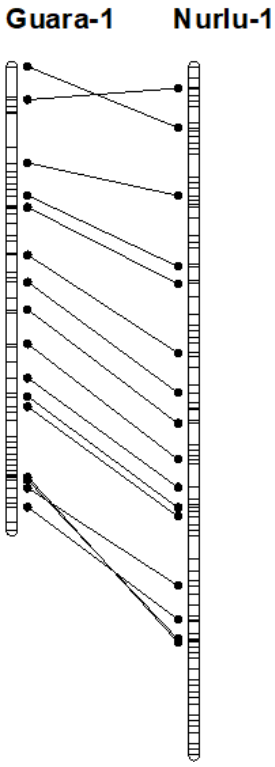

Figure 4.9. LG1 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2.2. LG2 in 'Guara x Nurlu' F1 population

LG2 of Guara map consists a total of 501 markers. Of them, 254 were DArT, 225 were SNP and 22 were SSR markers. LG2 map of Guara had a total map length of 52.87 cM. The average distance between the markers was 0.11 cM and the average marker density was 9.48 markers per cM. LG2 of the Nurlu cultivar included 407 (215 DArT, 172 SNP and 20 SSR) markers with a total map length of 57.24 cM. The average distance between markers was 0.14 cM and the average marker density was 7.11. In LG2 of Guara, 5.79% (13 DArT, 8 SNP and 8 SSR) of the markers were skewed markers, while 48 DArT, 13 SNP and 6 SSR (16.46%) markers showed skewed segregation in LG2 of Nurlu map (Figure 4.10, Table 4.3 and Table 4.4).

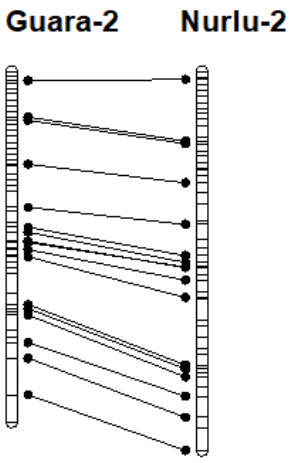

Figure 4.10. LG2 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2.3. LG3 in 'Guara x Nurlu' F1 population

LG3 of Guara map was constructed by 372 (163 DArT, 192 SNP and 17 SSR) markers with a total map length of 62.16 cM. The average distance between the markers was 0.17 cM and the average marker density was 5.98 markers per cM. LG3 of Nurlu cultivar included 117 DArT, 139 SNP and 14 SSR markers, and the

total map length was 55.96 cM. The average distance between markers was 0.21 cM and the average marker density was 4.82. The number of skewed markers in LG3 of Guara cultivar was 81 (21.77%), while LG3 of Nurlu map had 10 skewed markers with 3.70% (Figure 4.11, Table 4.3 and Table 4.4).

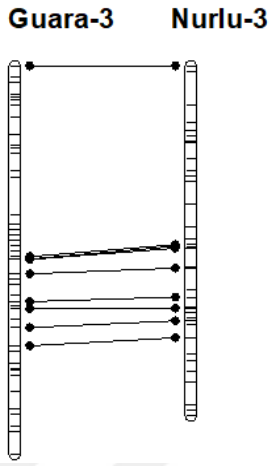

Figure 4.11. LG3 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2.4. LG4 in 'Guara x Nurlu' F1 population

LG4 of Guara map consists a total of 291 markers. Among them, 126 were DArT, 152 were SNP and 13 were SSR markers. LG4 map of Guara had a total map length of 51.71 cM. The average distance between the markers was 0.18 cM and the average marker density was 5.63 markers per cM. LG4 of the Nurlu cultivar included 369 (156 DArT, 201 SNP and 12 SSR) markers with a total map length of 56.33 cM. The average distance between markers was 0.15 cM and the average marker density was 6.55. In LG4 of Guara map, 29.55% (57 DArT, 28 SNP and 1 SSR) of the markers were skewed markers, while only two (0.54%; 1 DArT and 1 SSR) markers showed skewed segregation in LG4 of Nurlu map (Figure 4.12, Table 4.3 and Table 4.4).

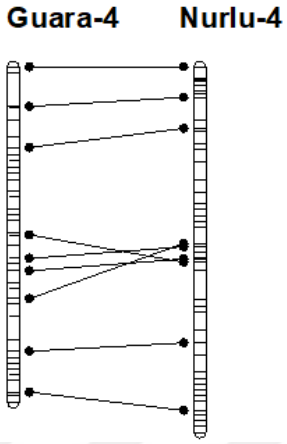

Figure 4.12. LG4 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2.5. LG5 in 'Guara x Nurlu' F1 population

LG5 of Guara was constructed by 250 (124 DArT, 115 SNP and 11 SSR) markers with a total map length of 54.15 cM. The average distance between the markers was 0.22 cM and the average marker density was 4.62 markers per cM. LG5 of Nurlu cultivar included 96 DArT, 139 SNP and 12 SSR markers, and the total map length was 57.55 cM. The average distance between markers was 0.23 cM and the average marker density was 4.29. The number of skewed markers in LG5 of Guara cultivar was seven (2.80%), while LG5 of Nurlu map had only one skewed marker (0.40%; Figure 4.13, Table 4.3 and Table 4.4).

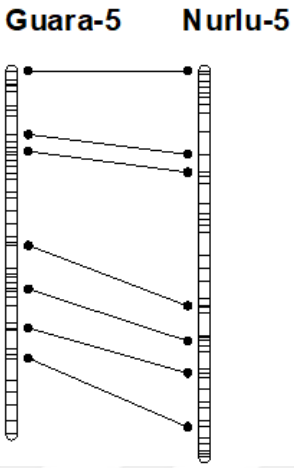

Figure 4.13. LG5 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2.6. LG6 in 'Guara x Nurlu' F1 population

LG6 of Guara consists a total of 70 markers. Of them, 27 were DArT, 28 were SNP and 15 were SSR markers. LG6 p of Guara had a total map length of 52.72 cM. The average distance between the markers was 0.75 cM and the average marker density was 1.33 markers per cM. LG6 of the Nurlu cultivar included 500 (182 DArT, 301 SNP and 17 SSR) markers with a total map length of 57.53 cM. The average distance between markers was 0.12 cM and the average marker density was 8.69. In LG6 of Guara, 54.29% (19 DArT, 6 SNP and 13 SSR) of the markers were skewed markers, while only 10 SSR (2.0%) markers had skewed segregation in LG6 of Nurlu map (Figure 4.14, Table 4.3 and Table 4.4).

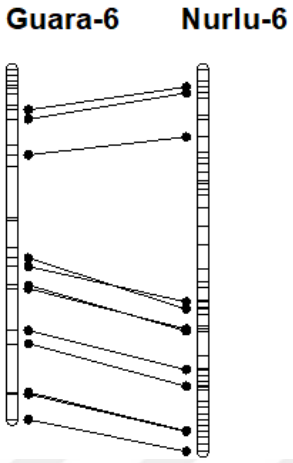

Figure 4.14. LG6 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2.7. LG7 in 'Guara x Nurlu' F1 population

LG7 of Guara map was constructed by 314 (143 DArT, 161 SNP and 10 SSR) markers with a total map length of 47.76 cM. The average distance between the markers was 0.15 cM and the average marker density was 6.57 markers per cM. LG7 of Nurlu cultivar included 133 DArT, 170 SNP and 15 SSR markers, and the total map length was 64.33 cM. The average distance between markers was 0.20 cM and the average marker density was 4.94. The number of skewed markers in LG7 of Guara cultivar was only six (1.91%), while LG7 of Nurlu map had only two (0.63%) skewed markers (Figure 4.15, Table 4.3 and Table 4.4).

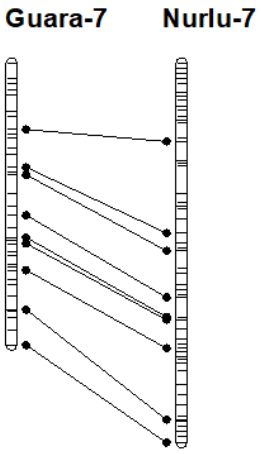

Figure 4.15. LG7 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

4.1.2.8. LG8 in 'Guara x Nurlu' F1 population

LG8 of Guara map included a total of 341 markers. Of them, 179 were DArT, 151 were SNP and 11 were SSR markers. LG8 map of Guara had a total map length of 49.43 cM. The average distance between the markers was 0.14 cM and the average marker density was 6.90 markers per cM. LG8 of the Nurlu cultivar included 414 (212 DArT, 188 SNP and 14 SSR) markers with a total map length of 58.55 cM. The average distance between markers was 0.14 cM and the average marker density was 7.07. In LG8 of Guara map, 35.48% (97 DArT, 23 SNP and 1 SSR) of the markers were skewed markers, while only three (0.72%; 2 DArT and 1 SSR) markers showed skewed segregation in LG8 of Nurlu map (Figure 4.16, Table 4.3 and Table 4.4).

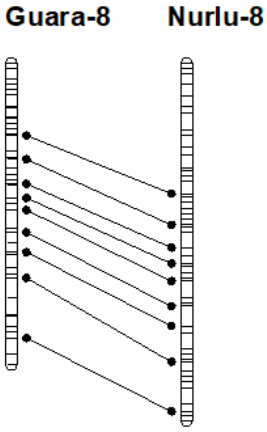

Figure 4.16. LG8 parental maps of 'Guara x Nurlu' F1 population. The lines represent the common SSR markers between two parental maps.

There are a few studies on the construction of genetic linkage maps in almond. Dirlewanger et al. (2004) constructed the *Prunus* reference map using 'Texas x Earlygold' F2 population. The reference map had a total length of 519 cM. Sanchez-Perez et al. (2007) constructed 'R1000 x Desmayo Largueta' genetic map with a total length of 395 cM. Almond linkage map constructed by Tavassolian et al. (2010) had a map length of 594.1 Cm. Fernandez i Marti et al. (2011) constructed a linkage map in 'Vivot x Blanquerna' F1 population with a total map length of 378.9 cM. Paizila (2016) constructed two consensus linkage maps using 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations, 'Gülcan-2 x Lauranne' map had a total length of 514.6 Cm, the 'Guara x Nurlu' linkage map had a total length of 493.9 cM.

In this study, we enriched the linkage maps of Paizila (2016) by SNP and DArT markers in the same population and constructed four parental linkage maps. Gülcan-2 map had a total length of 469.9 cM, Lauranne map had a map length of 572.2 cM, Guara map had covered a total length of 443.9 cM and Nurlu map had a total map length of 516 cM. All four genetic linkage maps we constructed in this study had similar total map lengths with the *Prunus* reference map.

Dirlewanger et al. (2004) constructed the *Prunus* reference map using 'Texas x Earlygold' F2 population and the average marker distance was 0.92 cM. Sanchez-Perez et al. (2007) constructed 'R1000 x Desmayo Largueta' genetic map and the average marker distance was 7.1 cM. There were 17 gaps larger than 10 cM in the linkage groups and the largest gap was 24 cM. An almond linkage map constructed by Tavassolian et al. (2010) with an average marker distance of 6.2 cM and there were 11 gaps that larger than 10 cM, and the largest gap was 21.9 cM. Fernandez i Marti et al. (2011) constructed a linkage map in 'Vivot x Blanquerna' F1 population and the average marker distance was 7.3 cM, there were 19 gaps larger than 10 cM and the largest gap was 28.6 cM. Paizila (2016) constructed two consensus linkage maps using 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations, 'Gülcan-2 x Lauranne' map had an average marker distance of 3.2 cM, and there were 8 gaps larger than 10 cM and the largest gap was 18.9 cM. The 'Guara x Nurlu' linkage map had an average marker distance of 3.2 cM, and there were only 4 gaps larger than 10 cM and the largest gap was 18.1 cM.

In this study, Gülcan-2 map had an average marker distance of 0.15 cM. Lauranne map had an average marker distance of 0.18cM. In Guara map the average marker distance was 0.24 cM and in Nurlu map the average marker distance was 0.17 cM. Only Lauranne map had one gap larger than 10 cM. The genetic linkage maps constructed in this study had much higher marker density than the other linkage maps of almond till date. We compared the linkage maps in this study with the high-density linkage maps constructed in other *Prunus* species. The average marker distance was 1.1 cM, 0.7 cM, 1.18, 1.62 cM and 1.5 cM in the studies performed by Klagger et al. (2013), Guajardo et al. (2015), Wang et al. (2015), Kitamura et al. (2018) and Nunez-Lilli et al. (2019), respectively. Therefore, the linkage maps constructed in this study are the most high-density linkage maps in almond so far.

In this study, Gülcan-2, Lauranne, Guara and Nurlu maps had a total of 714 (24.5%), 516 (15.4%), 383 (19.4%) and 189 (5.0%) distorted markers, respectively (Table 4.1, Table 4.2, Table 4.3 and Table 4.4). Factors such as chromosome loss,

genetic isolation mechanisms, and the presence of viability genes may be reason for the distortion. Non-biological factors such as scoring errors and also sampling errors can also lead to distortion in segregation ratios (Hong et al., 2010). DArT markers exhibited more distorted segregation than SNP markers in all maps. For example, distorted marker ratios of DArT markers (25.6% and 18.3%) in Gülcan-2 and Lauranne genetic maps were higher than SNP markers (10.6 and %6.4%), respectively. Similarly, distorted marker ratios of DArT markers (22.3% and 8.4%) in Guara and Nurlu genetic maps were higher than SNP markers (6.6½ and %3.1%), respectively (Table 4.1, Table 4.2, Table 4.3 and Table 4.4). This may due to the nature of the DArT markers, DArT markers are scored as presence or absence of a single allele due to whether the restriction enzyme can or cannot cut the region.

4.2. QTL Analysis

4.2.1. Correlation Analysis Among Traits

Shell L (SL), Shell a (Sa), Shell b (Sb), Kernel L (KL), Kernel a (Ka), Kernel b (Kb), Nut Length (NLh), Nut Width (NW), Nut Thickness (NT), Nut Weight (NWt), Kernel Length (KLh), Kernel Width (KW), Kernel Thickness (KT), Kernel Weight (KWt), Kernel Nut Ratio (KNR), Double Kernel Ratio (DKR), Softness of Shell (SoS), Nut Shape (NS), Shell Color Intensity (SCI), Kernel Color Intensity (KCI) and Shriveling of Kernel (SoK) of F1 individuals derived from two almond populations ‘Gülcan-2 x Lauranne’ and ‘Guara x Nurlu’ were phenotyped during four consecutive years (2015, 2016, 2017 and 2018). All the characters had significant variations within the F1 population.

The correlation statistics were given in Table 4.5, Table 4.6, Table 4.7, Table 4.8, Table 4.9, Table 4.10, Table 4.11, Table 4.12 for two F1 populations, and the correlation Tables were summarized in Table 13 for two populations and for four growing seasons to follow significantly correlated characters at $p = 0.01$.

Shell L and Shell b were ($p = 0.01$) positively correlated during three years in both populations. Shell a and Shell b were ($p = 0.01$) positively correlated during

four years in both populations. Kernel a had positive correlation with Shell b in at least two years in both populations. Kernel b also had positive correlations with Shell a, Shell b, Kernel L and Kernel b in at least two years in both populations.

Three nut dimensions (Nut Length, Nut Width, and Nut Thickness) had positive correlations between them as well as with Nut Weight, Kernel Weight, Kernel Length and Kernel Width during at least three years in both populations. Kernel Length was also positively correlated with Kernel Width and Kernel Weight during all four years in both populations. Kernel Length and Kernel Thickness were positively correlated during three years in 'Guara x Nurlu' population (Table 4.5, Table 4.6, Table 4.7, Table 4.8, Table 4.9, Table 4.10, Table 4.11, Table 4.12 and Table 4.13).

Kernel Nut Ratio was positively correlated with Kernel Thickness, Softness of Shell and Nut Shape during at least two years in both populations. Double Kernel Ratio was positively correlated with Nut Width, Nut Thickness and Nut Weight in three years at least in one of the populations. Nut Shape was positively correlated with Nut Length, Kernel Length and Kernel Nut Ratio during at least three years in one of the populations. Shell Color Intensity and Shell L were positively correlated during at least two years in both populations. Kernel Color Intensity was positively correlated with Kernel L, Kernel b and Shriveling of Kernel in at least two years in both populations (Table 4.5, Table 4.6, Table 4.7, Table 4.8, Table 4.9, Table 4.10, Table 4.11, Table 4.12 and Table 4.13).

Kernel Nut Ratio was negatively correlated with Shell a, Nut Width, Nut Thickness, Nut Weight, Kernel Width and Double Kernel Ratio during at least two years in one of the populations. Softness of Shell was negatively correlated with Nut Width and Nut Weight at least two years in both populations. Nut Shape was negatively correlated with Nut Width and Kernel Width during four growing seasons in 'Guara x Nurlu' population (Table 4.5, Table 4.6, Table 4.7, Table 4.8, Table 4.9, Table 4.10, Table 4.11 and Table 4.12).

In ‘Gülcan-2 x Lauranne’ population, Nut Weight and Kernel Weight, Nut Length and Kernel Length were correlated ($r \geq 0.8$) in all four growing seasons. Nut Width and Kernel Width, Nut Width and Nut Weight, Nut Length and Kernel Weight were significantly correlated ($r \geq 0.8$) in 2016, 2017 and 2018 growing seasons as well. Shell a and Shell b, Kernel a and Kernel b were significantly correlated ($r \geq 0.8$) in 2015 and 2016. Kernel Width and Kernel Weight, Nut Weight and Kernel Width were significantly correlated ($r \geq 0.8$) in 2016 and 2018. Other traits exhibited relatively low correlations or no significantly correlated (Table 4.5, Table 4.6, Table 4.7 and Table 4.8).

In ‘Guara x Nurlu’ population, Nut Width and Kernel Width were significantly correlated ($r \geq 0.8$) in 2015, 2016 and 2108 growing seasons. Nut Length and Kernel Length were significantly correlated ($r \geq 0.8$) in 2016, 2017 and 2018 growing seasons. Shell a and Shell b, Kernel a and Kernel b were significantly correlated ($r \geq 0.8$) in 2015 and 2016. Nut Width and Nut Weight, Kernel Width and Nut Weight were significantly correlated ($r \geq 0.8$) in 2016 and 2018. Other traits showed relatively low correlation or no significantly correlated (Table 4.9, Table 4.10, Table 4.11 and Table 4.12).

Table 4.5. The correlations between 21 different phenotypic characters related to kernel and nut traits in the 2015 growing season in 'Gülcan-2 x Lauranne' population.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCI	KCI	SoK
SL	1																				
Sa	.262*	1																			
Sb	.430**	.807**	1																		
KL	.287*	.445**	.467**	1																	
Ka	.350**	.276*	.414**	.467**	1																
kb	.280*	.411**	.476**	.494**	.848**	1															
NLh	.318**	.289*	.096	.318**	.065	.005	1														
NW	.246*	.206	.101	.316**	.207	.105	.588**	1													
NT	.034	.067	.040	.046	.025	-.114	.333**	.600**	1												
NWt	.118	.236*	.023	.242*	.025	.050	.622**	.698**	.385**	1											
KLh	.054	-.045	-.226*	.138	-.005	-.093	.846**	.432**	.229*	.553**	1										
KW	.014	.063	-.056	.161	.082	.011	.369**	.845**	.536**	.677**	.397**	1									
KT	-.349**	-.323**	-.311**	-.263*	-.040	-.068	-.165	-.106	.098	.009	.173	.190	1								
KWt	-.003	.167	-.046	.148	-.046	-.073	.657**	.574**	.461**	.764**	.660**	.590**	.270*	1							
KNR	-.213	-.180	-.133	-.201	-.136	-.165	-.171	-.492**	-.162	-.414**	.001	-.334**	.309**	.092	1						
DKR	.054	.205	.207	.132	-.028	-.008	.068	-.080	.295**	-.031	-.072	-.243*	-.185	.124	.102	1					
SoS	-.142	-.201	-.166	-.132	-.160	-.214	.038	-.156	-.014	-.271*	.085	-.272*	-.053	-.042	.285*	-.125	1				
NS	-.030	-.202	-.137	-.234*	-.104	-.117	-.400**	-.449**	-.222	-.343**	-.314**	-.310**	.158	-.298**	.362**	.029	.084	1			
SCI	.399**	.080	.313**	.243*	.177	.140	-.049	-.006	-.081	-.207	-.189	-.162	-.167	-.181	-.035	.185	.069	.052	1		
KCI	.017	.192	.142	.295**	.106	.255*	.026	.095	-.124	-.010	-.014	.147	.066	.063	.016	.103	-.228*	-.080	.404**	1	
SoK	.070	.170	.181	.119	.206	.257*	-.132	-.101	-.170	-.046	-.156	.028	.096	-.044	.015	-.038	-.224*	-.035	.070	.305**	1

* Correlation is significant at the $p = 0.05$ level (2-tailed). ** Correlation is significant at the $p = 0.01$ level (2-tailed).

Table 4.6. The correlations between 21 different phenotypic characters related to kernel and nut in the 2016 growing season in 'Gülcan-2 x Lauranne' population.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCI	KCI	SoK
SL	1																				
Sa	.252*	1																			
Sb	.577**	.814**	1																		
KL	.452**	.353**	.528**	1																	
Ka	.307**	.392**	.539**	.670**	1																
kb	.422**	.405**	.588**	.868**	.811**	1															
NLh	-0.098	0.145	-0.053	0.084	0.126	0.060	1														
NW	-0.050	0.008	-0.057	0.082	0.142	0.153	.680**	1													
NT	-0.176	-0.070	-0.100	-0.033	0.006	0.026	.517**	.694**	1												
NWt	-0.167	0.006	-0.113	-0.064	-0.049	-0.042	.777**	.856**	.820**	1											
KLh	-0.216	0.120	-0.121	0.005	0.056	-0.026	.927**	.612**	.428**	.736**	1										
KW	-0.163	-0.041	-0.115	-0.066	0.014	-0.004	.553**	.852**	.762**	.847**	.529**	1									
KT	-.335**	-.235*	-.288*	-0.113	-0.143	-0.048	0.116	.266*	.319**	.265*	0.188	.317**	1								
KWt	-0.207	-0.077	-0.189	-0.053	-0.016	-0.021	.757**	.802**	.707**	.893**	.799**	.800**	.472**	1							
KNR	0.028	-0.060	-0.023	0.129	0.148	0.141	-.257*	-.374**	-.485**	-.544**	-0.160	-.392**	.343**	-0.154	1						
DKR	0.145	-0.011	0.000	0.038	0.020	0.132	0.084	0.154	.381**	0.185	-0.019	0.219	0.005	0.073	-.254*	1					
SoS	.295*	-0.008	0.086	0.146	0.201	0.138	-0.078	-0.151	-0.163	-.289*	-0.120	-.245*	-0.007	-0.166	.351**	0.074	1				
NS	-.218*	0.065	-0.135	0.008	-0.028	-0.027	.295**	-0.087	-0.108	-0.020	.398**	-0.137	-0.002	0.079	.233*	0.002	-0.035	1			
SCI	.314**	0.074	.260*	.265*	.359**	.341**	0.058	0.145	0.072	0.072	0.005	0.114	-0.089	0.098	-0.007	.243*	0.086	-0.162	1		
KCI	.365**	.278*	.425**	.713**	.511**	.711**	0.097	0.120	-0.045	-0.028	0.043	-0.052	-.274*	-0.048	0.018	0.020	0.094	-0.048	.366**	1	
SoK	0.147	-0.123	0.026	0.222	.295*	.245*	-0.082	-0.070	-0.208	-0.156	-0.127	-0.164	0.020	-0.156	0.047	0.019	0.201	-0.190	0.028	0.120	1

* Correlation is significant at the $p = 0.05$ level (2-tailed). ** Correlation is significant at the $p = 0.01$ level (2-tailed).

Table 4.7. The correlations between 21 different phenotypic characters related to kernel and nut in the 2017 growing season in 'Gülcan-2 x Lauranne' population.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCl	KCl	SoK
SL	1																				
Sa	-.590**	1																			
Sb	.202	.345**	1																		
KL	.412**	-.244*	.267**	1																	
Ka	-.103	.132	.048	.208*	1																
kb	.402**	-.117	.448**	.722**	.264**	1															
NLh	-.247*	.382**	.042	.119	.099	.157	1														
NW	-.215*	.314**	.187	.162	.080	.123	.695**	1													
NT	-.109	.162	.139	-.126	-.105	-.150	.446**	.597**	1												
NWt	-.288**	.399**	.176	.173	-.025	.029	.778**	.816**	.628**	1											
KLh	-.318**	.312**	-.095	.018	.107	.009	.906**	.592**	.357**	.713**	1										
KW	-.319**	.253*	.052	.086	.050	-.032	.533**	.825**	.459**	.725**	.573**	1									
KT	-.224*	.049	-.013	.017	-.037	-.079	.138	.083	.156	.337**	.196	.289**	1								
KWt	-.279**	.282**	.061	.152	.019	.064	.825**	.742**	.502**	.907**	.795**	.723**	.507**	1							
KNR	.256*	-.431**	-.269**	-.079	.066	.058	-.337**	-.554**	-.524**	-.683**	-.255*	-.386**	.067	-.344**	1						
DKR	.379**	-.156	.365**	.029	-.264*	.059	-.216	-.245	.301*	-.172	-.300*	-.358**	-.184	-.319*	-.153	1					
SoS	.187	-.323**	-.143	-.265**	-.021	-.054	-.260*	-.423**	-.199	-.585**	-.187	-.374**	-.195	-.407**	.633**	.077	1				
NS	-.015	.119	.032	.077	.121	.126	.507**	.167	.013	.315**	.494**	.075	.004	.372**	-.113	-.201	-.136	1			
SCl	.417**	-.199	-.102	-.007	-.214*	.083	-.140	-.328**	-.195	-.319**	-.171	-.397**	-.258*	-.299**	.246*	.425**	.231*	-.071	1		
KCl	.137	.163	.352**	.326**	-.130	.430**	.296**	.333**	.099	.282**	.166	.210*	.122	.329**	-.044	.056	-.238*	-.098	.000	1	
SoK	-.192	.242*	.046	.230*	.152	.175	.278**	.404**	.045	.414**	.220*	.495**	.438**	.476**	-.128	-.331**	-.410**	-.038	-.157	.445**	1

* Correlation is significant at the $p = 0.05$ level (2-tailed). ** Correlation is significant at the $p = 0.01$ level (2-tailed).

Table 4.8. The correlations between 21 different phenotypic characters related to kernel and nut in the 2018 growing season in 'Gülcan-2 x Lauranne' population.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCI	KCI	SoK
SL	1																				
Sa	.082	1																			
Sb	,490**	,701**	1																		
KL	-.160	-.103	-.201	1																	
Ka	.120	.142	,246*	.123	1																
kb	-.140	.123	.006	,538**	,575**	1															
NLh	,261*	,537**	,487**	-,302**	,236*	,239*	1														
NW	-.074	,350**	,320**	-.187	,394**	,429**	,665**	1													
NT	-.113	,216*	.148	-.200	,295**	,208*	,525**	,691**	1												
NWt	.067	,484**	,462**	-,324**	,206*	,248*	,810**	,839**	,697**	1											
KLh	,258*	,447**	,410**	-,414**	.142	.158	,909**	,538**	,431**	,752**	1										
KW	-.096	,318**	,338**	-,218*	,308**	,340**	,470**	,805**	,610**	,703**	,383**	1									
KT	-.026	.063	-.029	-.194	-.025	-.037	.133	-.075	.130	.113	,277**	-.050	1								
KWt	.146	,416**	,390**	-,364**	.200	,254*	,854**	,695**	,604**	,866**	,894**	,585**	,416**	1							
KNR	.108	-,318**	-,316**	.074	-.093	-.103	-,267**	-,563**	-,390**	-,610**	-.102	-,474**	,385**	-.150	1						
DKR	.139	,213*	.145	.138	.138	.082	,227*	.114	,444**	.158	.139	.025	.020	.149	-.060	1					
SoS	.055	-,396**	-,301**	.066	.044	-.096	-.190	-,273**	-.049	-,433**	-.196	-.172	-.010	-,239*	,521**	.120	1				
NS	,338**	,238*	,214*	-.145	-.027	-.148	,403**	-,215*	-.105	.033	,392**	-,248*	.112	.197	,293**	.076	.198	1			
SCI	-,468**	,324**	-.029	-.122	-.031	,240*	,331**	,482**	,336**	,443**	,304**	,432**	.168	,370**	-,333**	-.066	-,242*	-.163	1		
KCI	.081	.033	.091	-,613**	.003	-,360**	.186	.132	.117	.175	,256*	.118	.046	.189	-.004	-,252*	.046	.138	.132	1	
SoK	-.088	-.011	-.065	,234*	-.114	.134	-,270**	-.155	-,340**	-,206*	-,291**	-.063	.056	-,243*	-.037	-,248*	-,216*	-.090	.136	-,344**	1

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

Table 4.9. The correlations between 21 different phenotypic characters related to kernel and nut in the 2015 growing season in 'Guara x Nurlu' population.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCI	KCI	SoK
SL	1																				
Sa	.222*	1																			
Sb	.457**	.845**	1																		
KL	.166	.447**	.421**	1																	
Ka	.090	.313**	.287**	.615**	1																
kb	.188	.409**	.373**	.666**	.837**	1															
NLh	.019	.143	.190	.345**	.214*	.305**	1														
NW	-.221*	-.033	-.153	.116	.014	.056	.543**	1													
NT	-.257**	-.011	-.140	-.015	-.130	-.082	.336**	.757**	1												
NWt	-.286**	.128	.026	.227*	.078	.178	.558**	.788**	.623**	1											
KLh	-.005	-.102	.014	.181	.178	.261**	.665**	.251*	.026	.445**	1										
KW	-.340**	-.108	-.228*	.170	.007	.044	.440**	.803**	.548**	.720**	.323**	1									
KT	-.203*	-.265**	-.253*	-.093	.011	.017	.022	-.065	.019	.043	.349**	.110	1								
KWt	-.261*	-.134	-.206	.170	.128	.162	.506**	.597**	.582**	.716**	.452**	.619**	.346**	1							
KNR	.032	-.316**	-.263*	-.043	.043	-.022	-.008	-.195	-.035	-.323**	.054	-.076	.447**	.411**	1						
DKR	.020	-.032	-.104	-.091	-.145	-.090	.086	.471**	.707**	.282**	-.250*	.205*	-.266**	.312**	.042	1					
SoS	.195	-.189	-.081	-.089	-.040	-.055	-.020	-.276**	-.177	-.343**	.210*	-.247*	.123	-.068	.366**	-.160	1				
NS	.078	.109	.049	-.018	-.021	.100	.062	-.343**	-.247*	-.159	.067	-.287**	.000	-.133	.007	-.050	.145	1			
SCI	.392**	-.208*	-.084	-.006	.030	.069	.045	-.223*	-.204*	-.357**	.073	-.136	.286**	-.083	.389**	-.154	.356**	.054	1		
KCI	.102	.114	.150	.400**	.230*	.370**	.211*	.005	-.102	.068	.192	.102	.350**	.118	.119	-.209*	.072	-.063	.395**	1	
SoK	-.105	-.235*	-.130	.056	.087	.115	.163	.066	-.071	.146	.219*	.216*	.455**	.294**	.272*	-.182	.091	-.061	.274**	.423**	1

* Correlation is significant at the $p = 0.05$ level (2-tailed). ** Correlation is significant at the $p = 0.01$ level (2-tailed).

Table 4.10. The correlations between 21 different phenotypic characters related to kernel and nut in the 2016 growing season in 'Guara x Nurlu' population.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DR	SoS	NS	SCl	KCl	SoK
SL	1																				
Sa	.393**	1																			
Sb	.625**	.800**	1																		
KL	.268*	.107	.232*	1																	
Ka	.163	.204	.389**	.624**	1																
kb	.208	.255*	.364**	.704**	.807**	1															
NLh	-.135	.058	.014	.317**	.056	.138	1														
NW	-.008	.131	.050	.094	-.118	-.090	.572**	1													
NT	-.047	.124	.088	.028	-.048	-.158	.487**	.741**	1												
NWt	-.150	.116	.003	.153	-.081	-.071	.722**	.882**	.779**	1											
KLh	-.174	-.057	-.091	.288**	.086	.171	.863**	.432**	.311**	.607**	1										
KW	.012	.053	-.016	.139	-.066	-.109	.521**	.841**	.545**	.755**	.451**	1									
KT	-.205	-.364**	-.325**	.068	.087	-.050	.149	.155	.259*	.145	.227*	.183	1								
KWt	-.182	-.116	-.153	.237*	.070	.013	.741**	.685**	.618**	.768**	.767**	.721**	.600**	1							
KNR	.038	-.343**	-.218*	.027	.176	.090	-.295**	-.550**	-.460**	-.636**	-.091	-.306**	.504**	-.016	1						
DKR	.068	.013	-.088	.061	-.067	-.118	.127	.356**	.576**	.388**	-.003	.244*	-.057	.177	-.420**	1					
SoS	.219*	-.178	-.092	.084	.165	.118	-.280**	-.356**	-.262*	-.438**	-.307**	-.289**	-.046	-.323**	.353**	.178	1				
NS	.010	-.163	-.073	.015	.080	.023	-.123	-.543**	-.314**	-.428**	-.109	-.459**	-.083	-.273**	.350**	-.243*	.277**	1			
SCl	.422**	.302**	.422**	.114	.178	.166	.021	.054	.154	-.033	-.041	.037	-.127	-.049	-.018	.111	.023	.009	1		
KCl	.161	.038	.092	.530**	.308**	.525**	.189	-.091	-.118	-.034	.145	-.001	.114	.103	.174	-.220*	.024	.029	.172	1	
SoK	.071	-.066	.029	.108	.080	.092	-.022	-.198	-.208	-.097	-.084	-.111	.041	-.079	.093	-.077	.125	.097	-.113	.315**	1

* Correlation is significant at the $p = 0.05$ level (2-tailed). ** Correlation is significant at the $p = 0.01$ level (2-tailed).

Table 4.11. The correlations between 21 different phenotypic characters related to kernel and nut in the 2017 growing season in 'Guara x Nurlu' population

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCl	KCl	SoK
SL	1																				
Sa	-.527**	1																			
Sb	.153	.310**	1																		
KL	.230*	-.153	.204*	1																	
Ka	-.161	.107	-.094	-.263**	1																
kb	.134	-.018	.200*	.851**	-.101	1															
NLh	-.041	.142	-.022	.011	.131	-.033	1														
NW	.064	-.006	.155	.239*	.013	.203*	.440**	1													
NT	-.134	.324**	-.056	-.306**	.188	-.274**	.060	-.310**	1												
NWt	-.352**	.423**	.131	.076	.203*	.120	.569**	.630**	.225*	1											
KLh	-.075	.073	-.052	.039	.188	.018	.832**	.296**	.002	.466**	1										
KW	-.213*	.315**	.031	.014	.328**	.094	.398**	.556**	.264**	.754**	.358**	1									
KT	-.054	-.045	-.098	.355**	.202*	.444**	.148	.205*	-.067	.347**	.270**	.249*	1								
KWt	-.221*	.201*	.007	.183	.366**	.272**	.575**	.510**	.122	.772**	.615**	.752**	.686**	1							
KNR	.306**	-.415**	-.215*	.148	.141	.188	-.124	-.293**	-.213*	-.535**	.069	-.185	.367**	.106	1						
DKR	.243*	-.070	.071	-.005	-.204	-.024	-.108	.236*	.234*	.119	-.294*	.053	-.044	-.067	-.394**	1					
SoS	.378**	-.358**	-.140	-.072	-.031	-.081	.118	-.077	-.090	-.447**	.124	-.263**	-.120	-.222*	.415**	.013	1				
NS	-.133	-.019	-.055	.055	-.068	-.026	.381**	-.267**	-.210*	-.174	.477**	-.291**	-.024	.017	.280**	-.502**	.186	1			
SCl	.510**	-.328**	.031	-.016	-.066	.000	.104	.038	-.121	-.295**	.100	-.144	-.009	-.069	.371**	.050	.420**	.043	1		
KCl	.083	-.060	.042	.607**	-.123	.621**	.050	.051	-.293**	.020	.093	-.018	.390**	.180	.239*	-.288*	-.013	.135	.161	1	
SoK	-.006	.126	.081	.400**	-.076	.397**	.067	.050	-.096	.183	.056	.199*	.366**	.303**	.147	-.087	-.207*	.004	-.019	.620**	1

* Correlation is significant at the $p = 0.05$ level (2-tailed). ** Correlation is significant at the $p = 0.01$ level (2-tailed).

Table 4.12. The correlations between 21 different phenotypic characters related to kernel and nut in the 2018 growing season in 'Guara x Nurlu' population.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCl	KCl	SoK
SL	1																				
Sa	-.096	1																			
Sb	.317**	.675**	1																		
KL	.048	-.205	-.085	1																	
Ka	.060	.380**	.384**	.166	1																
kb	.081	.328**	.312**	.366**	.734**	1															
NLh	.177	.316**	.199	-.144	.317**	.486**	1														
NW	.007	.331**	.305**	-.253*	.146	.412**	.616**	1													
NT	-.051	.108	.186	-.294**	-.041	.095	.437**	.769**	1												
NWt	-.047	.353**	.335**	-.245*	.211*	.408**	.676**	.899**	.816**	1											
KLh	.206	.179	.139	-.041	.418**	.605**	.857**	.473**	.304**	.554**	1										
KW	.002	.334**	.287*	-.206*	.293**	.513**	.571**	.890**	.640**	.834**	.515**	1									
KT	.092	.091	.106	.020	.523**	.255*	.127	.009	.048	.126	.279**	.179	1								
KWt	.125	.310**	.268*	-.158	.535**	.569**	.664**	.614**	.475**	.690**	.750**	.748**	.678**	1							
KNR	.258*	-.174	-.171	.123	.316**	.064	-.146	-.521**	-.553**	-.550**	.105	-.282**	.616**	.206*	1						
DKR	-.054	-.124	-.043	-.018	-.291**	-.149	.017	.289**	.544**	.321**	-.033	.186	-.261*	-.020	-.490**	1					
SoS	.162	-.243*	-.338**	.002	-.067	-.115	.032	-.244*	-.247*	-.424**	.034	-.245*	-.028	-.120	.473**	-.153	1				
NS	.159	-.034	-.125	.134	.120	-.021	.199	-.438**	-.307**	-.242*	.224*	-.342**	.180	.017	.349**	-.264**	.219*	1			
SCl	-.417**	.222*	-.056	.015	-.106	.107	.092	.212*	.150	.200*	.008	.190	-.005	.076	-.262*	-.054	-.163	-.169	1		
KCl	-.089	.036	-.038	-.514**	-.356**	-.551**	-.148	.011	.229*	.018	-.267**	-.106	-.202	-.215*	-.283**	.183	.009	-.041	-.002	1	
SoK	-.052	-.169	-.020	-.176	-.291**	-.165	.013	.173	.379**	.132	-.064	.006	-.379**	-.170	-.384**	.416**	.005	-.080	.041	.265**	1

* Correlation is significant at the $p = 0.05$ level (2-tailed). ** Correlation is significant at the $p = 0.01$ level (2-tailed).

Table 4.13. The correlations between 21 different phenotypic characters related to kernel and nut during four growing season in two F1 populations.

	SL	Sa	Sb	KL	Ka	kb	NLh	NW	NT	NWt	KLh	KW	KT	KWt	KNR	DKR	SoS	NS	SCl	KCl	SoK
SL	1																				
Sa	-/-	1																			
Sb	$3^a/3^b$	$4^a/4^b$	1																		
KL	$2^a/-$	$2^a/1^b$	$3^a/1^b$	1																	
Ka	$2^a/-$	$1^a/2^b$	$2^a/3^b$	$2^a/2^b$	1																
kb	$2^a/-$	$2^a/2^b$	$3^a/3^b$	$4^a/4^b$	$4^a/3^b$	1															
NLh	$1^a/-$	$2^a/1^b$	$1^a/-$	$1^a/2^b$	$-/1^b$	$-/2^b$	1														
NW	-/-	$2^a/1^b$	$1^a/1^b$	$1^a/-$	$1^a/-$	$1^a/1^b$	$4^a/4^b$	1													
NT	-/-	$-/1^b$	-/-	-/-	$1^a/-$	-/-	$4^a/3^b$	$4^a/4^b$	1												
NWt	-/-	$2^a/2^b$	$1^a/1^b$	-/-	-/-	$-/1^b$	$4^a/4^b$	$4^a/4^b$	$4^a/3^b$	1											
KLh	-/-	$2^a/-$	$1^a/-$	$-/1^b$	$-/1^b$	$-/2^b$	$4^a/4^b$	$4^a/4^b$	$4^a/2^b$	$4^a/4^b$	1										
KW	-/-	$1^a/1^b$	$1^a/-$	-/-	$1^a/2^b$	$1^a/1^b$	$4^a/4^b$	$4^a/4^b$	$4^a/4^b$	$4^a/4^b$	$4^a/4^b$	1									
KT	-/-	-/-	-/-	$-/1^b$	$-/1^b$	$-/1^b$	-/-	-/-	$1^a/-$	$1^a/1^b$	$1^a/3^b$	$2^a/-$	1								
KWt	-/-	$2^a/1^b$	$1^a/-$	-/-	$-/2^b$	$-/2^b$	$4^a/4^b$	$4^a/4^b$	$4^a/3^b$	$4^a/4^b$	$4^a/4^b$	$4^a/4^b$	$3^a/4^b$	1							
KNR	-/-	-/-	-/-	-/-	$-/1^b$	-/-	-/-	-/-	-/-	-/-	-/-	-/-	$3^a/4^b$	$-/1^b$	1						
DKR	-/-	-/-	$1^a/-$	-/-	-/-	-/-	-/-	$-/3^b$	$3^a/3^b$	$-/3^b$	-/-	-/-	-/-	$-/1^b$	-/-	1					
SoS	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	$3^a/4^b$	-/-	1				
NS	-/-	-/-	-/-	-/-	-/-	-/-	$3^a/1^b$	-/-	-/-	$1^a/-$	$3^a/1^b$	-/-	-/-	$-/1^b$	$2^a/3^b$	-/-	$-/1^b$	1			
SCl	$3^a/2^b$	$1^a/1^b$	$1^a/1^b$	-/-	$1^a/-$	$1^a/-$	$1^a/-$	$1^a/-$	$1^a/-$	$1^a/-$	$1^a/-$	$1^a/-$	$-/1^b$	$-/1^b$	$-/2^b$	$1^a/-$	$-/2^b$	-/-	1		
KCl	$1^a/-$	-/-	$2^a/-$	$3^a/3^b$	$1^a/1^b$	$2^a/3^b$	$1^a/-$	$1^a/-$	-/-	$1^a/-$	-/-	-/-	$-/2^b$	$-/1^b$	-/-	-/-	-/-	-/-	$2^a/1^b$	1	
SoK	-/-	-/-	-/-	$-/1^b$	-/-	$1^a/-$	$1^a/-$	$1^a/-$	$-/1^b$	$1^a/-$	-/-	$1^a/-$	$1^a/2^b$	$1^a/2^b$	-/-	$-/1^b$	-/-	-/-	$-/1^b$	$2^a/4^b$	1

[a] Gülcan-2 x Luranne, [b] Guara x Nurlu, [-] No significant correlation detected at the $p = 0.01$ level.

4.2.2. Frequency Distribution of the Traits

The frequency distribution analysis of SL, Sa, Sb, KL, Ka, Kb, NLh, NW, NT, NWt, KLh, KW, KT, KWt, KNR, DKR, SoS, NS, SCI, KCI and SoK of F1 individuals derived from two almond populations 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' were done during four consecutive years. The mean, maximum, minimum values and standard deviation of all the traits were given in Table 4.14 and Table 4.15.

Table 4.14. Mean values, maximum and minimum values, standard deviation of all traits evaluated in 2015, 2016, 2017 and 2018 in 'Gülcan-2 x Lauranne' population.

Trait	Year	F1 Progenies				Parents	
		Mean	Max	Min	SD	Gülcan-2	Lauranne
SL	2015	42.2	50.4	34.2	3.3	44.1	49.3
	2016	41.0	51.2	33.2	4.1	36.5	45.7
	2017	53.3	67.0	35.7	5.2	39.4	57.9
	2018	47.1	57.8	39.7	3.3	48.8	53.0
Sa	2015	10.2	13.5	7.6	1.1	10.0	10.6
	2016	10.9	13.0	8.3	1.0	10.1	9.1
	2017	8.3	12.3	4.8	1.3	8.3	7.6
	2018	11.2	13.5	8	1.1	11.7	11.2
Sb	2015	23.9	33.3	19.0	2.6	26.1	24.5
	2016	24.6	29.2	17.3	2.3	24.0	22.4
	2017	26.4	30.8	21.8	2.0	28.8	24.7
	2018	25.2	30.6	20.2	2.1	27.6	23.6
KL	2015	34.8	43.0	28.0	3.2	36.2	33.0
	2016	35.6	48.4	28.1	3.9	26.3	31.7
	2017	45.2	55.2	29.8	4.8	48.6	51.8
	2018	46.0	50	38.8	2.1	42.5	46.5
Ka	2015	14.6	17.0	11.0	1.2	15.5	15.0
	2016	15.2	18.4	12.4	1.5	13.2	12.0
	2017	14.8	17.5	9.5	1.4	14.6	14.1
	2018	16.0	17.7	14.5	0.7	15.7	16.8
kb	2015	26.7	33.5	19.0	2.9	26.4	26.0

	2016	26.5	34.5	19.9	3.4	19.1	25.0
	2017	31.5	42.8	22.1	4.2	34.0	35.6
	2018	25.3	28.9	21.2	1.8	24.1	27.0
NLh	2015	33.1	44.1	26.1	2.9	33.3	33.4
	2016	32.0	39.4	26.0	2.9	24.2	32.7
	2017	31.8	41.8	24.5	3.5	31.1	32.8
	2018	32.5	42.9	26	3.1	34.7	33.9
NW	2015	21.0	26.4	17.1	1.8	22.2	21.3
	2016	20.8	26.9	17.3	2.1	16.6	19.3
	2017	21.1	27.3	16.4	2.0	20.9	20.5
	2018	20.7	26	16.5	1.7	23.7	21.6
NT	2015	13.5	15.7	11.3	1.0	13.9	14.1
	2016	13.1	16.2	10.4	1.3	10.7	15.2
	2017	13.9	16.0	11.8	0.9	13.4	13.8
	2018	13.4	16.2	10.9	1.1	14.5	14.0
NWt	2015	2.7	5.2	1.4	0.7	3.9	1.9
	2016	3.5	5.7	2.0	0.9	1.8	2.1
	2017	3.0	6.1	1.6	0.9	3.3	2.8
	2018	3.2	5.5	1.9	0.7	4.0	3.3
KLh	2015	22.8	28.3	18.0	2.0	23.7	23.1
	2016	22.2	26.3	17.6	2.0	17.0	21.5
	2017	22.9	29.6	17.9	2.3	23.2	23.6
	2018	23.3	29.4	19.1	2.1	26.0	24.5
KW	2015	11.9	14.8	9.3	1.1	14.0	12.3
	2016	11.5	14.8	9.0	1.3	9.5	10.4
	2017	12.6	15.6	8.9	1.3	13.6	12.3
	2018	12.3	15.8	10.3	1.0	14.8	12.7
KT	2015	5.1	6.3	3.5	0.6	5.4	5.4
	2016	6.4	7.5	5.1	0.5	5.7	5.5
	2017	6.3	8.0	4.7	0.6	6.9	7.2
	2018	5.9	7.4	4.7	0.4	6.7	6.6
KWt	2015	0.6	1.0	0.3	0.1	1.0	0.6
	2016	0.8	1.4	0.5	0.2	0.5	0.7
	2017	0.8	1.4	0.4	0.2	0.9	0.9
	2018	0.8	1.2	0.52	0.1	1.1	0.9

KNR	2015	23.3	33.8	16.6	3.4	25.9	31.1
	2016	24.1	31.1	17.7	2.4	25.1	33.5
	2017	28.2	39.5	20.0	4.4	28.8	33.6
	2018	24.6	33.5	17.4	2.9	27.8	28.5
DKR	2015	10.0	83.3	0.0	17.6	0.0	0.0
	2016	10.2	100.0	0.0	19.5	0.0	0.0
	2017	16.6	90.0	0.0	20.5	3.3	10.0
	2018	17.1	86.7	0	21.0	3.3	20.0
SoS	2015	4.0	7	1	1.8	1	7
	2016	4.3	7	1	1.3	3	3
	2017	5.1	9	1	2.1	3	5
	2018	3.8	7	1	1.6	3	5
NS	2015	6.4	9	3	1.3	5	5
	2016	6.0	9	3	1.6	7	7
	2017	5.9	9	1	2.1	7	7
	2018	5.1	9	3	1.5	7	7
SCl	2015	5.1	7	3	1.6	7	7
	2016	4.4	7	1	1.5	7	7
	2017	4.6	7	3	2.0	5	5
	2018	6.1	7	1	1.7	5	7
KCl	2015	4.4	9	3	1.3	3	3
	2016	5.5	9	3	2.0	3	3
	2017	4.2	9	1	1.9	3	5
	2018	5.2	7	3	1.4	3	5
SoK	2015	4.0	7	1	2.2	5	1
	2016	3.8	7	1	2.0	1	1
	2017	5.5	7	1	1.7	5	1
	2018	5.8	7	1	1.5	5	1

Table 4.15. Mean values, maximum and minimum values, standard deviation of all traits evaluated in 2015, 2016, 2017 and 2018 in 'Guara x Nurlu' population.

Trait	Year	F1 Progenies				Parents	
		Mean	Max	Min	SD	Guara	Nurlu
SL	2015	45.0	55.2	35.4	4.5	48.6	40.6
	2016	43.3	54.1	26.6	4.6	44.3	53.5
	2017	57.6	68.9	38.4	5.3	65.2	42.5
	2018	49.9	57.6	43.8	2.5	54.7	52.9
Sa	2015	10.6	15.9	6.3	1.6	10.9	9.8
	2016	11.4	13.9	7.9	1.2	11.0	11.7
	2017	8.7	12.8	5.6	1.3	8.3	10.9
	2018	12.4	15.0	10.0	1.0	13.5	12.2
Sb	2015	25.2	38.9	12.9	4.0	27.2	22.7
	2016	24.3	29.3	15.2	3.0	22.5	27.9
	2017	27.5	35.3	22.8	2.3	28.3	23.6
	2018	27.6	32.2	23.3	1.9	28.4	28.1
KL	2015	37.5	48.9	29.3	3.9	35.5	41.4
	2016	35.7	43.3	28.9	2.9	35.3	39.1
	2017	49.9	64.5	30.5	6.1	51.4	40.3
	2018	45.6	48.7	40.0	1.7	43.9	47.3
Ka	2015	15.2	19.6	10.2	1.6	16.0	16.7
	2016	15.4	18.5	12.2	1.3	11.9	16.1
	2017	15.0	17.7	11.3	1.2	16.1	16.3
	2018	16.7	20.1	14.8	1.0	16.9	17.4
Kb	2015	28.7	45.8	18.8	4.0	31.0	32.3
	2016	26.5	34.5	20.1	2.8	21.5	28.5
	2017	34.8	47.8	24.7	4.9	39.1	29.7
	2018	27.6	33.9	22.8	2.4	27.3	30.7
NLh	2015	36.2	44.6	30.4	2.8	34.4	31.1
	2016	35.9	41.4	29.7	2.7	42.9	40.3
	2017	33.4	41.2	27.2	2.5	31.4	35.0
	2018	35.8	45.0	29.8	2.8	36.2	34.9
NW	2015	22.3	27.4	17.7	2.1	22.0	21.3
	2016	22.4	27.9	17.5	2.3	27.9	25.7

	2017	20.6	25.1	13.7	2.6	21.6	20.9
	2018	22.4	29.9	17.8	2.0	23.3	22.3
NT	2015	16.2	20.4	13.1	1.4	15.0	13.9
	2016	15.3	19.9	11.5	1.4	18.8	17.9
	2017	16.0	22.4	12.9	1.9	14.2	15.8
	2018	16.0	20.8	12.9	1.3	16.2	16.1
NWt	2015	3.4	5.5	2.0	0.7	2.9	3.6
	2016	4.8	7.7	2.8	1.1	6.4	6.2
	2017	3.3	5.6	2.0	0.8	2.4	4.9
	2018	4.5	9.5	2.7	1.1	4.4	5.8
KLh	2015	24.7	31.5	20.3	2.1	23.1	24.6
	2016	25.3	29.8	21.7	1.8	26.9	29.5
	2017	24.5	30.0	19.9	1.8	22.8	27.1
	2018	26.1	31.0	22.4	1.8	26.3	27.7
KW	2015	12.3	15.6	9.8	1.2	12.8	10.9
	2016	12.3	15.7	9.7	1.2	14.3	15.6
	2017	12.4	15.2	10.1	1.1	12.8	12.4
	2018	13.5	18.0	11.1	1.2	14.2	14.2
KT	2015	5.6	7.3	3.8	0.7	5.5	7.2
	2016	6.6	7.9	5.4	0.6	7.5	7.8
	2017	6.5	8.2	5.2	0.7	6.3	7.5
	2018	6.2	8.2	4.5	0.7	6.5	7.5
KWt	2015	0.9	1.7	0.5	0.2	0.9	0.9
	2016	1.1	1.5	0.7	0.2	1.2	1.3
	2017	0.9	1.5	0.6	0.2	0.9	1.2
	2018	1.1	2.0	0.7	0.2	1.1	1.4
KNR	2015	25.8	33.1	13.9	4.0	30.9	25.6
	2016	22.7	31.3	15.6	3.2	24.8	20.9
	2017	29.0	43.4	17.7	4.7	35.3	24.6
	2018	24.1	36.0	14.5	4.4	25.7	24.3
DKR	2015	32.9	100.0	0.0	29.4	20.0	0.0
	2016	10.3	100.0	0.0	19.0	36.7	0.0
	2017	22.7	100.0	0.0	25.3	16.7	13.3
	2018	41.8	100.0	0.0	30.4	66.7	0.0
SoS	2015	3.9	9	1	2.0	5	3

	2016	3.3	7	1	1.4	3	3
	2017	5.1	9	1	2.2	5	3
	2018	3.8	7	1	1.7	5	3
NS	2015	5.9	9	3	1.5	5	9
	2016	6.2	9	3	1.6	5	9
	2017	6.3	9	3	2.6	5	7
	2018	6.0	9	1	1.7	5	7
SCl	2015	6.7	9	3	1.7	9	7
	2016	5.9	9	1	2.0	7	7
	2017	7.0	9	3	1.6	9	5
	2018	6.0	9	1	2.1	7	7
KCl	2015	5.5	9	3	1.6	7	7
	2016	5.5	9	1	1.6	5	7
	2017	5.9	9	1	2.2	3	7
	2018	4.9	9	1	2.1	3	7
SoK	2015	4.2	7	1	2.1	5	7
	2016	4.4	7	1	2.0	5	5
	2017	5.9	7	1	1.5	5	5
	2018	3.8	7	1	2.4	5	5

4.2.2.1. Shell L (SL)

The Shell L (SL) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The SL value of cv. Gülcan-2 was 44.1, 36.5, 39.4 and 48.8 in 2015, 2016, 2017 and 2018, respectively, whereas they were 49.3, 45.7, 57.9 and 53.0 in Lauranne. The ranges of SL values varied from 34.2 to 50.4, 33.2 to 51.2, 35.7 to 67.0 and 39.7 to 57.8 in 2015, 2016, 2017 and 2018, respectively. The average SL in 2015, 2016, 2017 and 2018 were 42.2, 41.0, 53.3 and 47.1, respectively (Table 4.14). The frequency distribution of SL in 2015, 2016, and 2017 had a good fit to normal distribution (Table 4.14 and Figure 4.17).

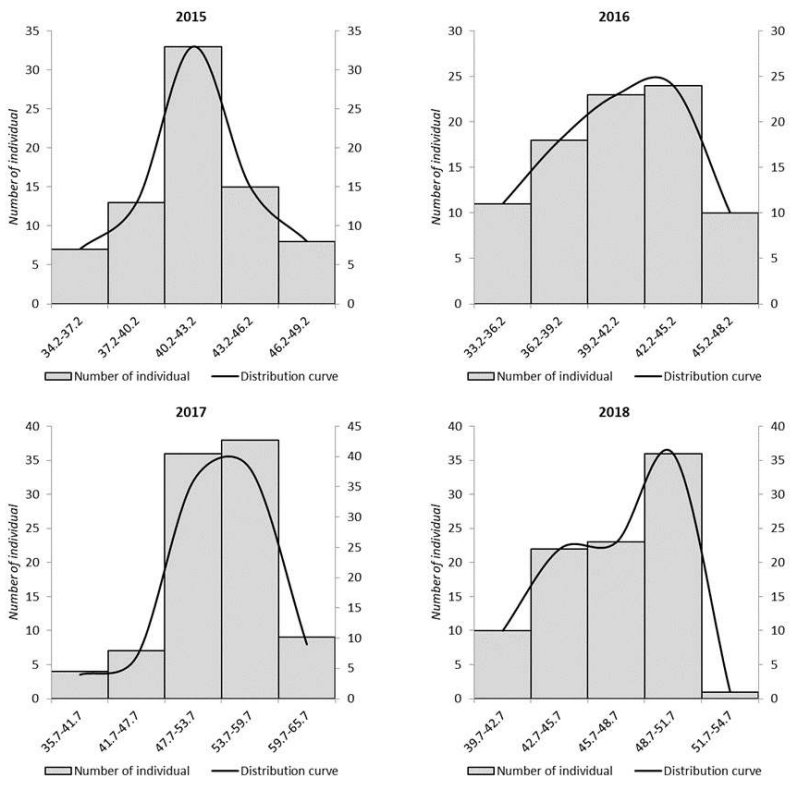

Figure 4.17. Frequency distributions of Shell L (SL) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.2. Shell a (Sa)

The Shell a (Sa) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The Sa value of cv. Gülcan-2 was 10.0, 10.1, 8.3 and 11.7 in 2015, 2016, 2017 and 2018, respectively, whereas they were 10.6, 9.1, 7.6 and 11.2 in Lauranne. The ranges of Sa values varied from 7.6 to 13.5, 8.3 to 13.0, 4.8 to 12.3 and 8.0 to 13.5 in 2015, 2016, 2017 and 2018, respectively. The average Sa for 2015, 2016, 2017 and 2018 were 10.2, 10.9, 8.3 and 11.2, respectively (Table 4.14). The frequency distribution of Sa values in four years had a good fit to normal distribution (Table 4.14 and Figure 4.18).

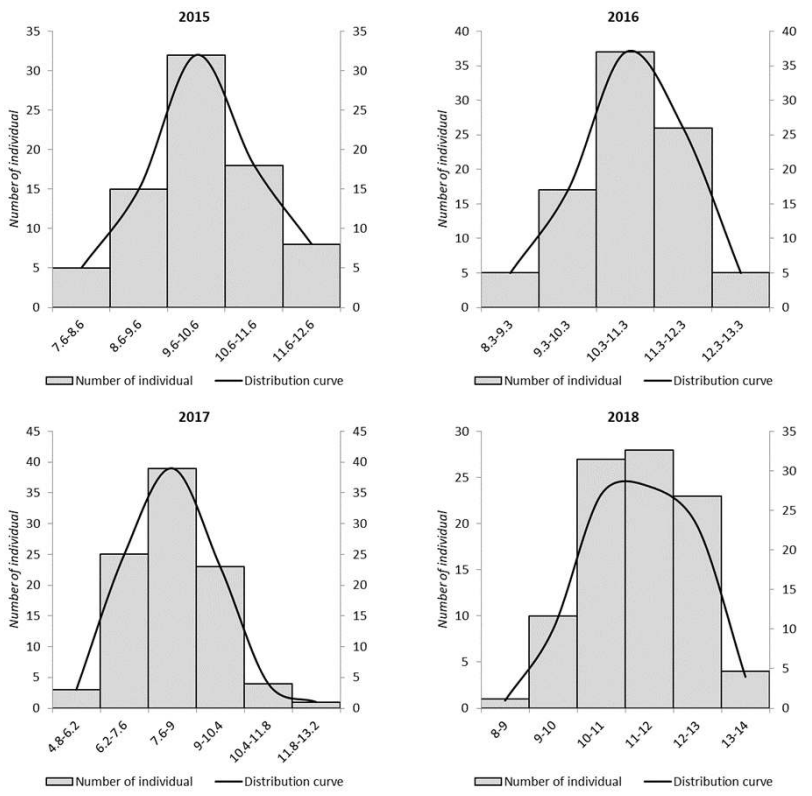

Figure 4.18. Frequency distributions of Shell a (Sa) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.3. Shell b (Sb)

The Shell b (Sb) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The Sb value of cv. Gülcan-2 was 26.1, 24.0, 28.8 and 27.6 in 2015, 2016, 2017 and 2018, respectively, whereas they were 24.5, 22.4, 24.7 and 23.6 in Lauranne. The ranges of Sb values varied from 19.0 to 33.3, 17.3 to 29.2, 21.8 to 30.8 and 20.2 to 30.6 in 2015, 2016, 2017 and 2018, respectively. The average Sb in 2015, 2016, 2017 and 2018 were 23.9, 24.6, 26.4 and 25.2, respectively (Table 4.14). The frequency distribution of Sb values for four years had a good fit to normal distribution (Table 4.14 and Figure 4.19).

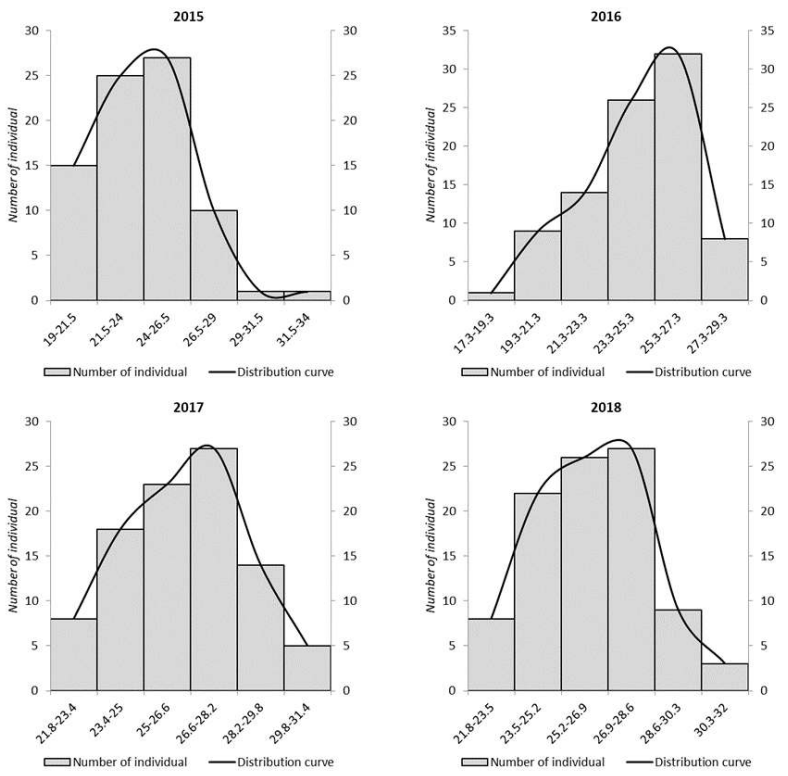

Figure 4.19. Frequency distributions of Shell b (Sb) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.4. Kernel L (KL)

The Kernel L (KL) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The KL value of cv. Gülcan-2 was 36.2, 26.3, 48.6 and 42.5 in 2015, 2016, 2017 and 2018, respectively, whereas they were 33.0, 31.7, 51.8 and 46.5 in Lauranne. The ranges of KL values varied from 28.0 to 43.0, 28.1 to 48.4, 29.8 to 55.2 and 38.8 to 50.0 in 2015, 2016, 2017 and 2018, respectively. The average KL in 2015, 2016, 2017 and 2018 were 34.8, 35.6, 45.2 and 46.0, respectively (Table 4.14). The frequency distribution of four years of KL values had a good fit to normal distribution (Table 4.14 and Figure 4.20).

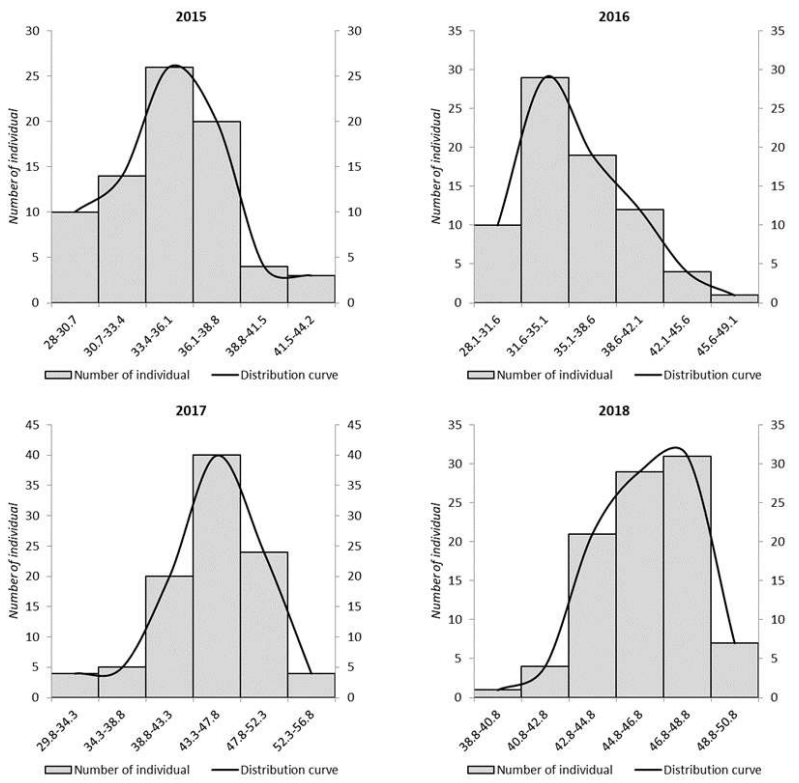

Figure 4.20. Frequency distributions of Kernel L (KL) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.5. Kernel a (Ka)

The Kernel a (Ka) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The Ka values of cv. Gülcan-2 were 15.5, 13.2, 14.6 and 15.7 in 2015, 2016, 2017 and 2018, respectively, whereas they were 15.0, 12.0, 14.1 and 16.8 in Lauranne. The ranges of Ka values varied from 11.0 to 17.0, 12.4 to 18.4, 9.5 to 17.5 and 14.5 to 17.7 in 2015, 2016, 2017 and 2018, respectively. The average Ka in 2015, 2016, 2017 and 2018 were 14.6, 15.2, 14.8 and 16.0, respectively (Table 4.14). The frequency distribution of Ka values had a good fit to normal distribution in four years (Table 4.14 and Figure 4.21).

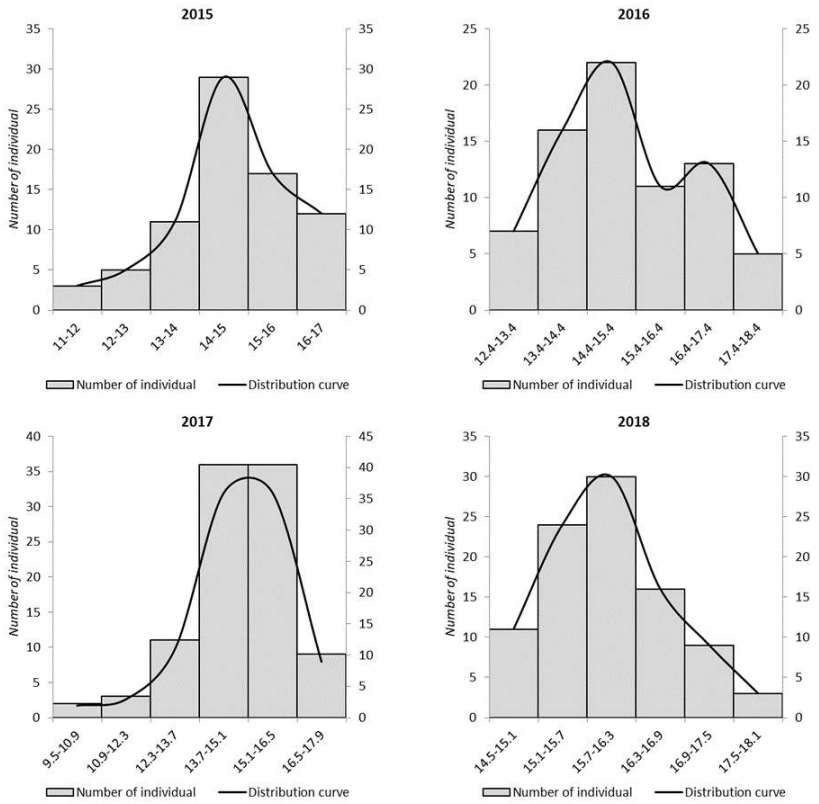

Figure 4.21. Frequency distributions of Kernel a (Ka) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.6. Kernel b (Kb)

The Kernel b (Kb) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The Kb values of cv. Gülcan-2 were 26.4, 19.1, 34.0 and 24.1 in 2015, 2016, 2017 and 2018, respectively, whereas they were 26.0, 25.0, 35.6 and 27.0 in Lauranne. The ranges of Kb values varied from 19.0 to 33.5, 19.9 to 34.5, 22.1 to 42.8 and 21.2 to 28.9 in 2015, 2016, 2017 and 2018, respectively. The average Kb in 2015, 2016, 2017 and 2018 were 26.7, 26.5, 31.5 and 25.3, respectively (Table 4.14). The frequency distribution of Kb values had a good fit to normal distribution in four years (Table 4.14 and Figure 4.22).

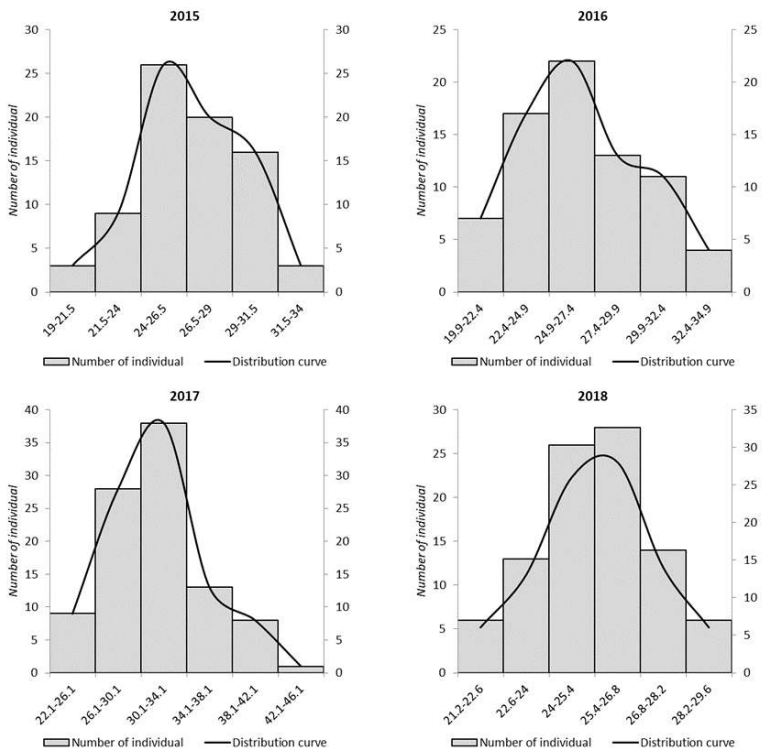

Figure 4.22. Frequency distributions of Kernel b (Kb) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.7. Nut Length (NLh)

The Nut Length (NLh) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The NLh value of cv. Gülcan-2 was 33.3 cm, 24.2 cm, 31.1 cm and 34.7 cm in 2015, 2016, 2017 and 2018, respectively, whereas they were 33.4 cm, 32.7 cm, 32.8 cm and 33.9 cm in Lauranne. The ranges of NLh values varied from 26.1 to 44.1 cm, 26.0 to 39.4 cm, 24.5 to 41.8 cm and 26.0 to 42.9 cm in 2015, 2016, 2017 and 2018, respectively. The average NLh in 2015, 2016, 2017 and 2018 were 33.1 cm, 32.0 cm, 31.8 cm and 32.5 cm, respectively. The standard deviation of NLh in 2015, 2016, 2017 and 2018 were 2.9, 2.9, 3.5 and 3.1, respectively (Table 4.14). The frequency distribution of NLh in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (as shown in Table 4.14 and Figure 4.23).

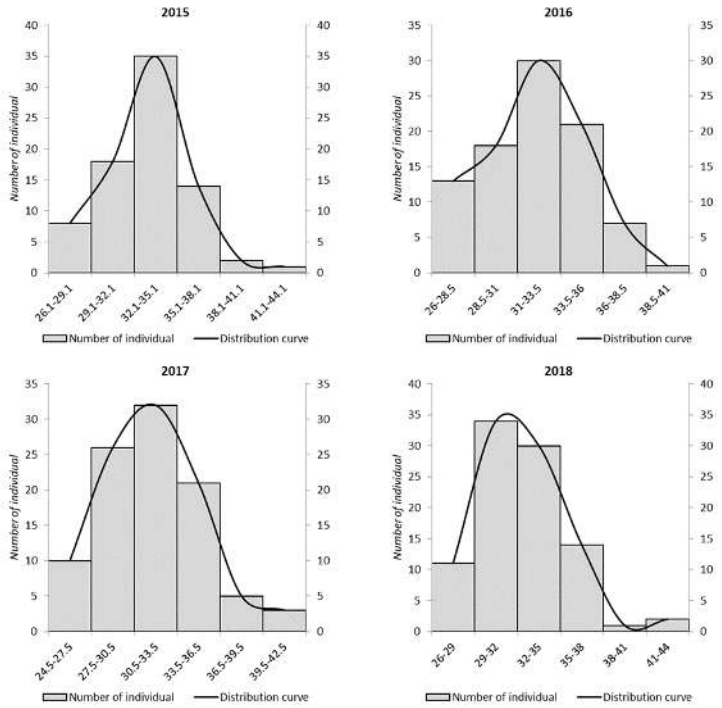

Figure 4.23. Frequency distributions of Nut Length (NL) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.8. Nut Width (NW)

The Nut Width (NW) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The NW value of cv. Gülcan-2 was 22.2 cm, 16.6 cm, 20.9 cm and 23.7 cm in 2015, 2016, 2017 and 2018, respectively, whereas they were 21.3 cm, 19.3 cm, 20.5 cm and 21.6 cm in Lauranne. The ranges of NW values varied from 17.1 to 26.4 cm, 17.3 to 26.9 cm, 16.4 to 27.3 cm and 16.5 to 26.0 cm in 2015, 2016, 2017 and 2018, respectively. The average NW in 2015, 2016, 2017 and 2018 were 21.0 cm, 20.8 cm, 21.1 cm and 20.7 cm, respectively. The standard deviation of NW in 2015, 2016, 2017 and 2018 were 1.8, 2.1, 2.0 and 1.7, respectively (Table 4.14). The frequency distribution of NW in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (as shown in Table 4.14 and Figure 4.24).

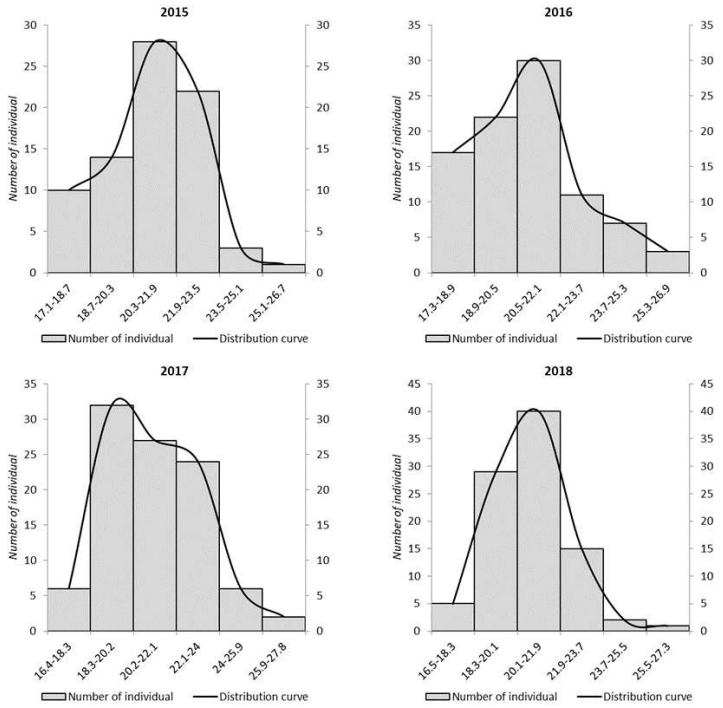

Figure 4.24. Frequency distributions of Nut Width (NW) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.9. Nut Thickness (NT)

The Nut Thickness (NT) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The NT value of cv. Gülcan-2 was 13.9 cm, 10.7 cm, 13.4 cm and 14.5 cm in 2015, 2016, 2017 and 2018, respectively, whereas they were 14.1 cm, 15.2 cm, 13.8 cm and 14.0 cm in Lauranne. The ranges of NT values varied from 11.3 to 15.7 cm, 10.4 to 16.2 cm, 11.8 to 16.0 cm and 10.9 to 16.2 cm in 2015, 2016, 2017 and 2018, respectively. The average NT in 2015, 2016, 2017 and 2018 were 13.5 cm, 13.1 cm, 13.9 cm and 13.4 cm, respectively. The standard deviation of NT in 2015, 2016, 2017 and 2018 were 1.0, 1.3, 0.9 and 1.1, respectively (Table 4.14). The frequency distribution of NT in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (as shown in Table 4.14 and Figure 4.25).

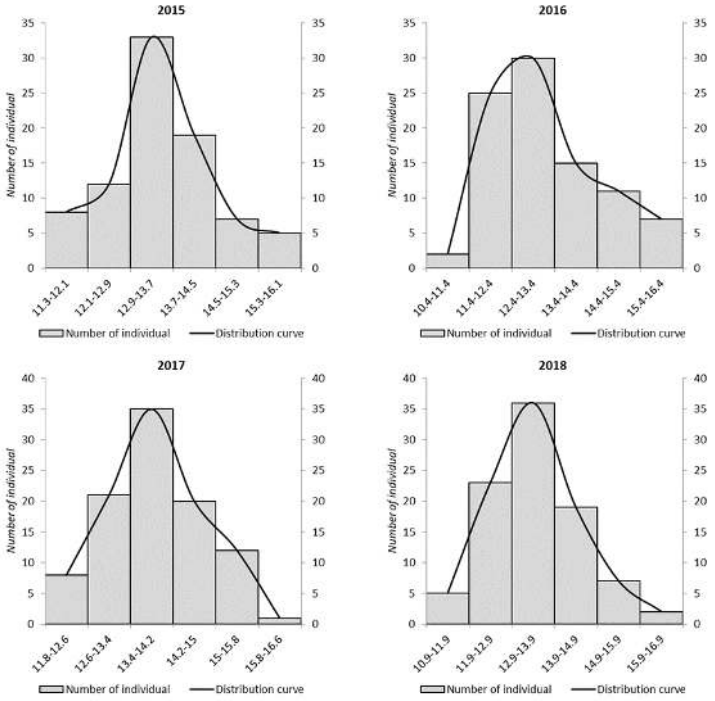

Figure 4.25. Frequency distributions of Nut Thickness (NT) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.10. Nut Weight (NWt)

The Nut Weight (NWt) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The NWt value of cv. Gülcan-2 was 3.9 g, 1.8 g, 3.3 g and 4.0 g in 2015, 2016, 2017 and 2018, respectively, whereas they were 1.9 g, 2.1 g, 2.8 g and 3.3 g in Lauranne. The ranges of NWt values varied from 1.4 to 5.2 g, 2.0 to 5.7 g, 1.6 to 6.1 g and 1.9 to 5.5 g in 2015, 2016, 2017 and 2018, respectively. The average NWt in 2015, 2016, 2017 and 2018 were 2.7 g, 3.5 g, 3.0 g and 3.2 g, respectively (Table 4.14). The frequency distribution of NWt in 2015, 2016 and 2018 had a good fit to normal distribution, while showed a skewed right distribution in 2017 (as shown in Table 4.14 and Figure 4.26).

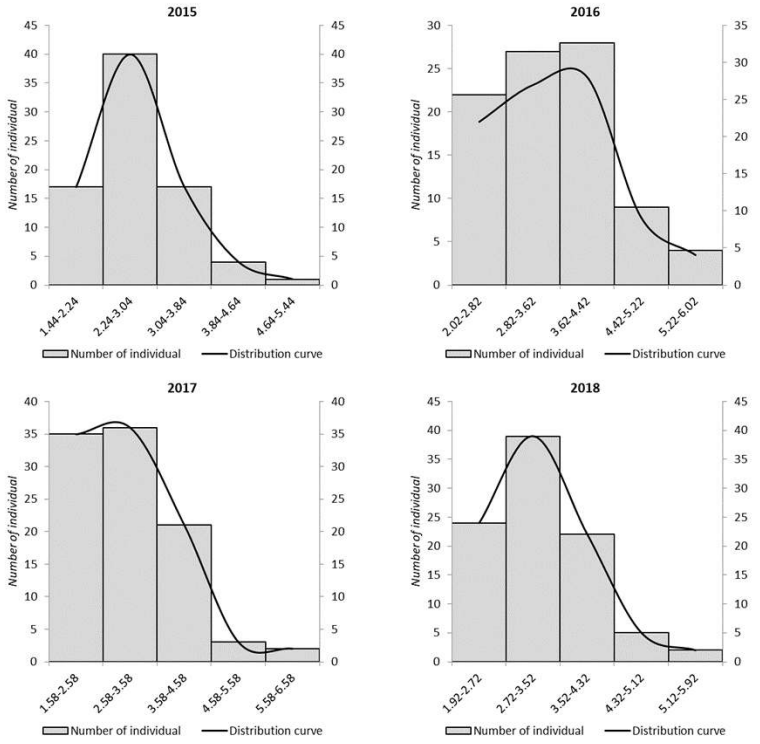

Figure 4.26. Frequency distributions of Nut Weight (NWt) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.11. Kernel Length (KLh)

The Kernel Length (KLh) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The KLh value of cv. Gülcan-2 was 23.7 cm, 17.0 cm, 23.2 cm and 26.0cm in 2015, 2016, 2017 and 2018, respectively, whereas they were 23.1 cm, 21.5 cm, 23.6 cm and 24.5cm in Lauranne. The ranges of KLh values varied from 18.0 to 28.3 cm, 17.6 to 26.3 cm, 17.9 to 29.6 cm and 19.1 to 29.4cm in 2015, 2016, 2017 and 2018, respectively. The average KLh in 2015, 2016, 2017 and 2018 were 22.8 cm, 22.2 cm, 22.9 cm and 23.3cm, respectively. The standard deviation of KLh in 2015, 2016, 2017 and 2018 were 2.0, 2.0, 2.3 and 2.1, respectively (Table 4.14). The frequency distribution of KLh in in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.14 and Figure 4.27).

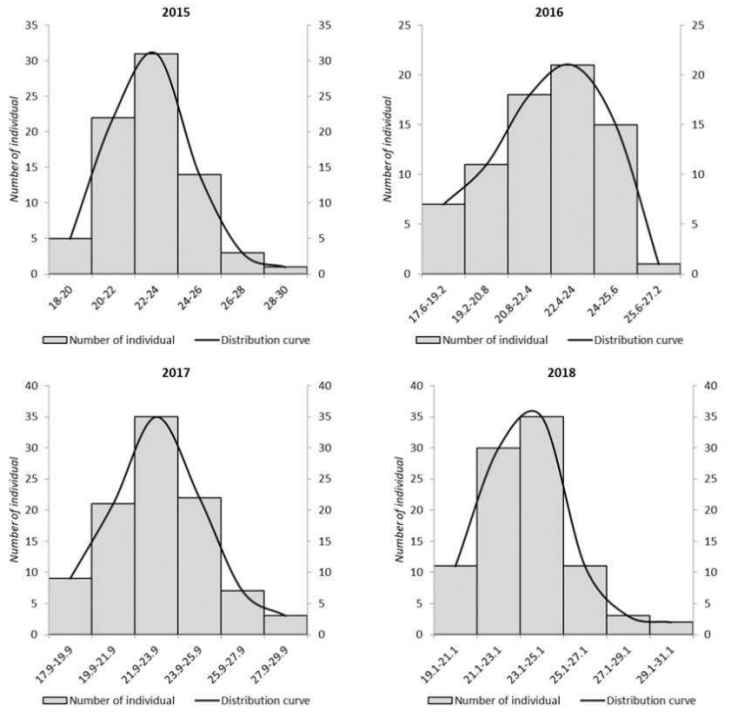

Figure 4.27. Frequency distributions of Kernel Lenght (KL) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.12. Kernel Width (KW)

The Kernel Width (KW) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The KW value of cv. Gülcan-2 was 14.0 cm, 9.5 cm, 13.6 cm and 14.8cm in 2015, 2016, 2017 and 2018, respectively, whereas they were 12.3 cm, 10.4 cm, 12.3 cm and 12.7cm in Lauranne. The ranges of KW values varied from 9.3 to 14.8 cm, 9.0 to 14.8 cm, 8.9 to 15.6 cm and 10.3 to 15.8cm in 2015, 2016, 2017 and 2018, respectively. The average KW in 2015, 2016, 2017 and 2018 were 11.9 cm, 11.5 cm, 12.6 cm and 12.3cm, respectively. The standard deviation of KW in 2015, 2016, 2017 and 2018 were 1.1, 1.3, 1.3 and 1.0, respectively (Table 4.14). The frequency distribution of KW in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.14 and Figure.4.28).

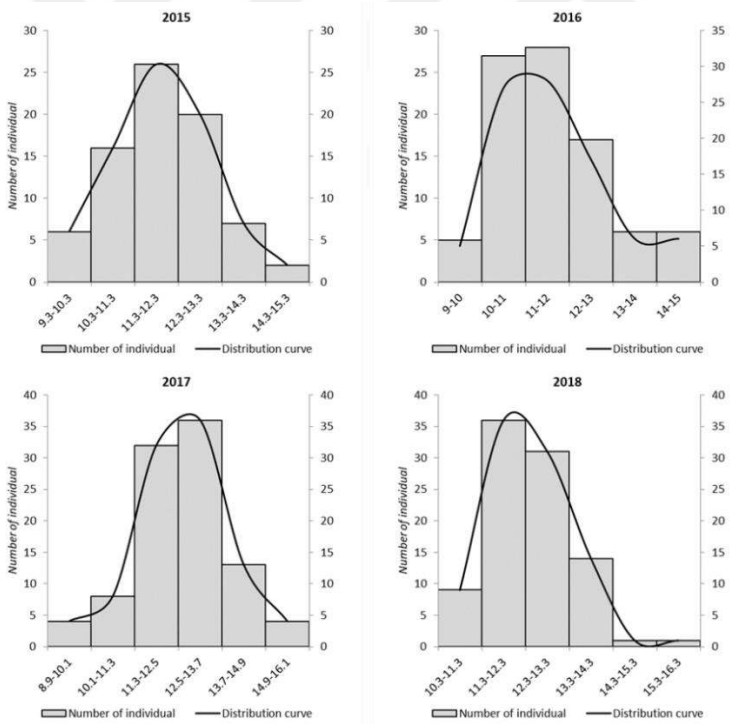

Figure 4.28. Frequency distributions of Kernel Width (KW) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.13. Kernel Thickness (KT)

The Kernel Thickness (KT) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The KT of value cv. Gülcan-2 was 5.4 cm, 5.7 cm, 6.9 cm and 6.7cm in 2015, 2016, 2017 and 2018, respectively, whereas they were 5.4 cm, 5.5 cm, 7.2 cm and 6.6cm in Lauranne. The ranges of KT values varied from 3.5 to 6.3 cm, 5.1 to 7.5 cm, 4.7 to 8.0 cm and 4.7 to 7.4 cm in 2015, 2016, 2017 and 2018, respectively. The average KT in 2015, 2016, 2017 and 2018 were 5.1 cm, 6.4 cm, 6.3 cm and 5.9 cm, respectively. The standard deviation of KT in 2015, 2016, 2017 and 2018 were 0.6, 0.5, 0.6 and 0.4, respectively (Table 4.14). The frequency distribution of KT in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.14 and Figure 4.29).

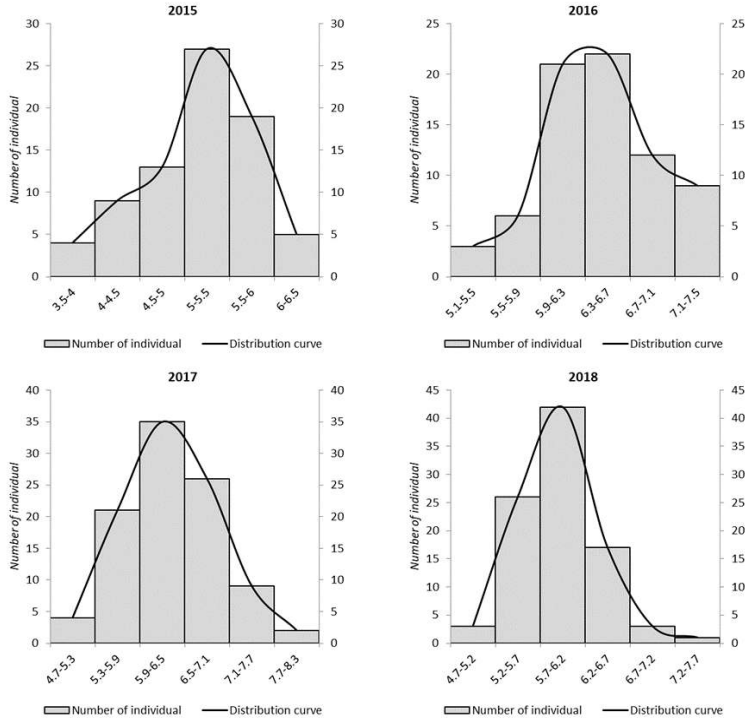

Figure 4.29. Frequency distributions of Kernel Thickness (KT) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.14. Kernel Weight (KWt)

The Kernel Weight (KWt) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The KWt value of cv. Gülcan-2 was 1.0 g, 0.5 g, 0.9 g and 1.1 g in 2015, 2016, 2017 and 2018, respectively, whereas they were 0.6 g, 0.7 g, 0.9 g and 0.9 g in Lauranne. The ranges of KWt values varied from 0.3 to 1.0 g, 0.5 to 1.4 g, 0.4 to 1.4 g and 0.5 to 1.2 g in 2015, 2016, 2017 and 2018, respectively. The average KWt in 2015, 2016, 2017 and 2018 were 0.6 g, 0.8 g, 0.8 g and 0.8 g, respectively (Table 4.14). The frequency distribution of KWt in 2015, 2016, 2017 and 2018 showed a good fit to normal distribution in all four years (Table 4.14 and Figure 4.30).

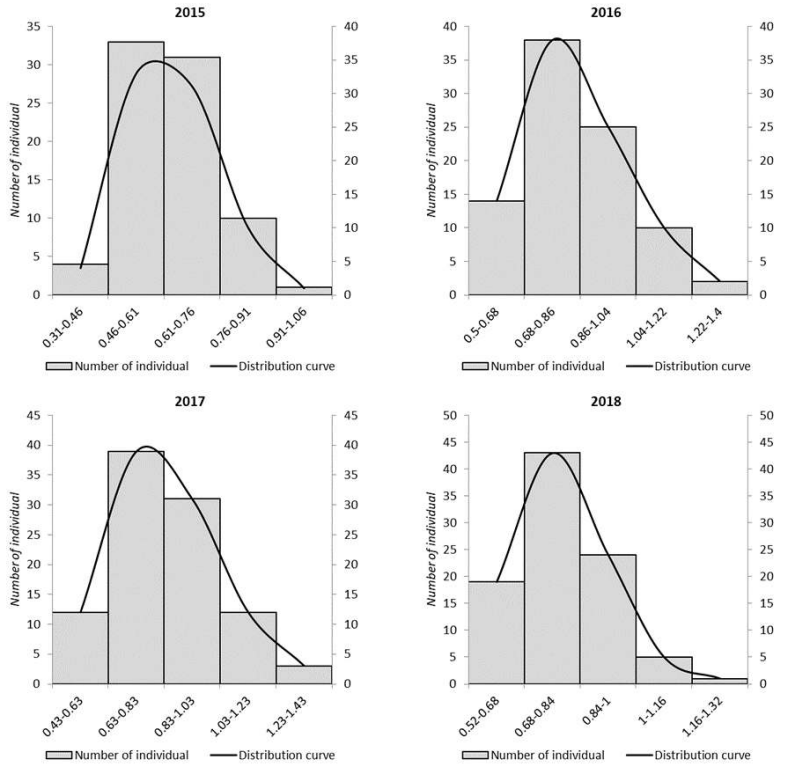

Figure 4.30. Frequency distributions of Kernel Weight (KW) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.15. Kernel Nut Ratio (KNR)

The Kernel Nut Ratio (KNR) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The KNR value of cv. Gülcan-2 was 25.9%, 25.1%, 28.8% and 27.8% in 2015, 2016, 2017 and 2018, respectively, whereas they were 31.1%, 33.5%, 33.6% and 28.5% in Lauranne. The ranges of KNR values varied from 16.6 to 33.8%, 17.7 to 31.1%, 20.0 to 39.5% and 17.4 to 33.5% in 2015, 2016, 2017 and 2018, respectively. The average KNR in 2015, 2016, 2017 and 2018 were 23.3%, 24.1%, 28.2% and 24.6%, respectively. The standard deviation of KNR in 2015, 2016, 2017 and 2018 were 3.4, 2.4, 4.4 and 2.9, respectively (Table 4.14). The frequency distribution of KNR in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.14 and Figure 4.31).

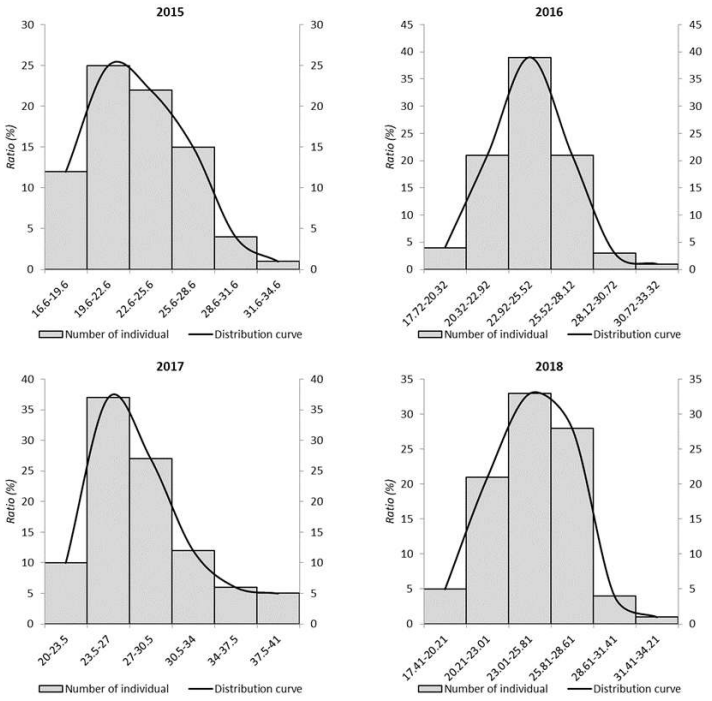

Figure 4.31. Frequency distributions of Kernel Nut Ratio (KNR) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.16. Double Kernel Ratio (DKR)

The Double Kernel Ratio (DKR) value of the parents and F1 population of 'Gülcan-2 x Lauranne' were measured. The DKR value of cv. Gülcan-2 was 0%, 0%, 3.3% and 3.3% in 2015, 2016, 2017 and 2018, respectively, whereas they were 0%, 0%, 10.0% and 20.0% in Lauranne. The ranges of DKR values varied from 0 to 83.3%, 0 to 100%, 0 to 90% and 0 to 86.7% in 2015, 2016, 2017 and 2018, respectively. The average DKR in 2015, 2016, 2017 and 2018 were ranged from 10.0%, 10.2%, 16.6% and 17.1%, respectively (Table 4.14). The frequency distribution of KNR in 2015, 2016, 2017 and 2018 showed a good fit to skewed right distribution (Table 4.14 and Figure 4.32).

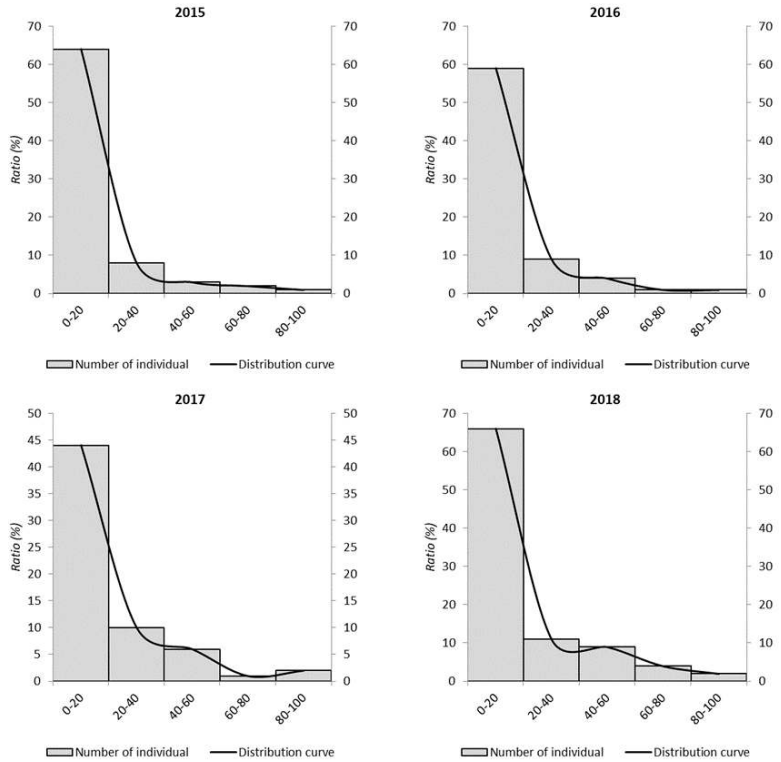

Figure 4.32. Frequency distributions of Double Kernel Ratio (DKR) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.17. Softness of Shell (SoS)

Softness of Shell (SoS) values in 'Gülcan-2 x Lauranne' population varied from extremely hard (1), hard (3), intermediate (5), soft (7) and paper (9) in each year. The SoS value of cv. Gülcan-2 scored as hard in 2016, 2017 and 2018 years and scored as extremely hard in 2018. Lauranne was scored as intermediate in 2017 and 2018, while scored as soft and hard in 2015 and 2016. Standard deviation of four years was 1.8, 1.3, 2.1 and 1.6, respectively (Table 4.14). The distribution of SoS in 2015, 2016 and 2018 assessed as normal distribution, while SosS value showed bimodal distribution in 2017. (Table 4.14 and Figure 4.33).

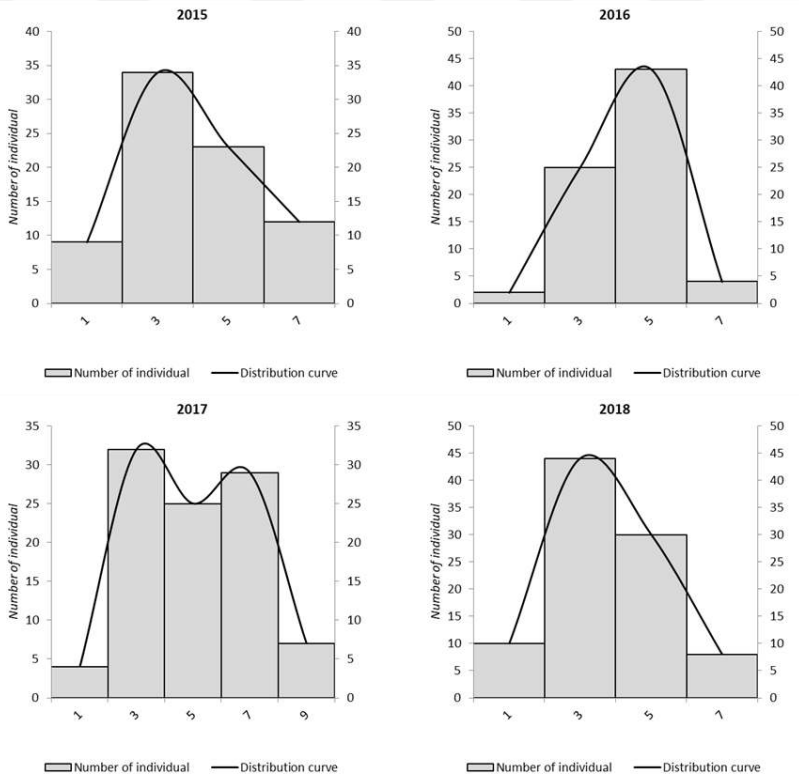

Figure 4.33. Frequency distributions of Softness of Shell (SoS) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.18. Nut Shape (NS)

Nut Shape (NS) values in 'Gülcan-2 x Lauranne' population varied from round (1), ovate (3), oblong (5), cordate (7) and extremely narrow (9) in each year. According to the observations, the NS values of two parents Gülcan-2 and Lauranne were similar. The NS value of cv. Gülcan-2 scored as cordate in 2016, 2017 and 2018 years and scored as oblong in 2015. Lauranne was also scored as cordate in 2016, 2017 and 2018 years and scored as oblong in 2015. The standard deviation of NS in 2015, 2016, 2017 and 2018 were 1.3, 1.6, 2.1 and 1.5, respectively (Table 4.14). The frequency distribution of NS in 2015, 2016 and 2018 assessed as normal distribution, while NS showed a bimodal distribution in 2017 (Table 4.14 and Figure 4.34).

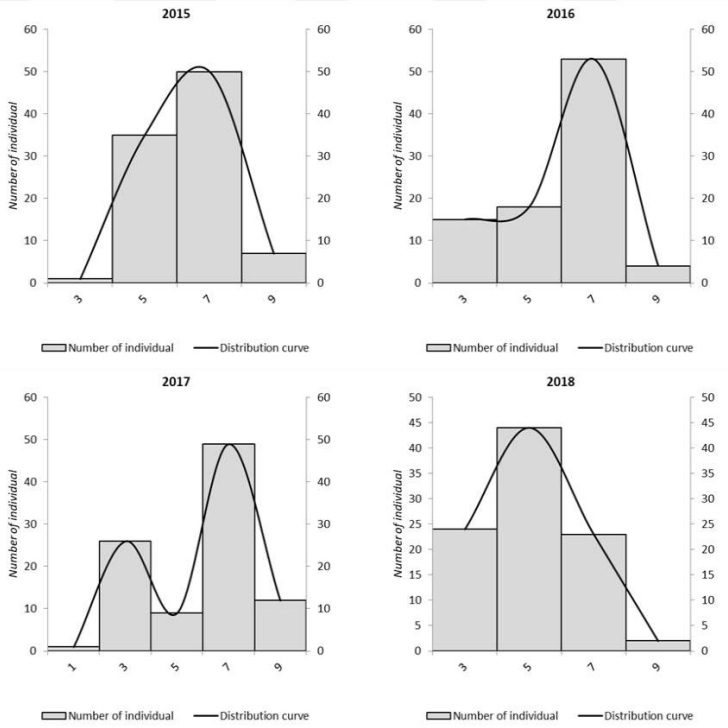

Figure 4.34. Frequency distributions of Nut Shape (NS) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.19. Shell Color Intensity (SCI)

Shell Color Intensity (SCI) values in 'Gülcan-2 x Lauranne' population varied from extremely light (1), light (3), intermediate (5), dark (7) and extremely dark (9) in each year. According to the observations, the SCI values of two parents Gülcan-2 and Lauranne were similar. The SCI value of cv. Gülcan-2 scored as dark in year 2015 and 2016, and scored as intermediate in year 2017 and 2018. Lauranne was also scored as dark in 2015, 2016 and 2018 years and scored as intermediate in 2015. The standard deviation of SCI in 2015, 2016, 2017 and 2018 were 1.6, 1.5, 2.0 and 1.7, respectively (Table 4.14). The distribution of SCI in 2015, 2016 and 2018 assessed as normal distribution, while SCI value showed bimodal distribution in 2017 (Table 4.14 and Figure 4.35).

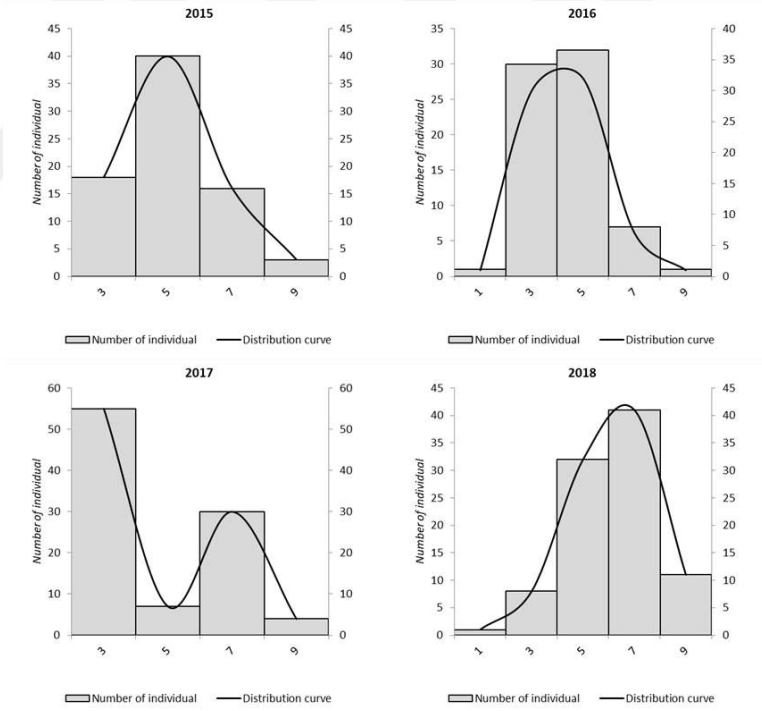

Figure 4.35. Frequency distributions of Shell Color Intensity (SCI) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.20. Kernel Color Intensity (KCI)

Khell Color Intensity (KCI) values in 'Gülcan-2 x Lauranne' population varied from extremely light (1), light (3), intermediate (5), dark (7) and extremely dark (9) in each year. According to the observations, the KCI values of two parents Gülcan-2 and Lauranne were similar. The KCI value of cv. Gülcan-2 scored as light in all four years. Lauranne was also scored as light in 2015 and 2016, while scored as intermediate in 2017 and 2018. The standard deviation of KCI in 2015, 2016, 2017 and 2018 were 1.3, 2.0, 1.9 and 1.4, respectively (Table 4.14). The distribution of KCI in 2015, 2016 and 2018 assessed as normal distribution, while KCI value showed bimodal distribution in 2017 (Table 4.14 and Figure 4.36).

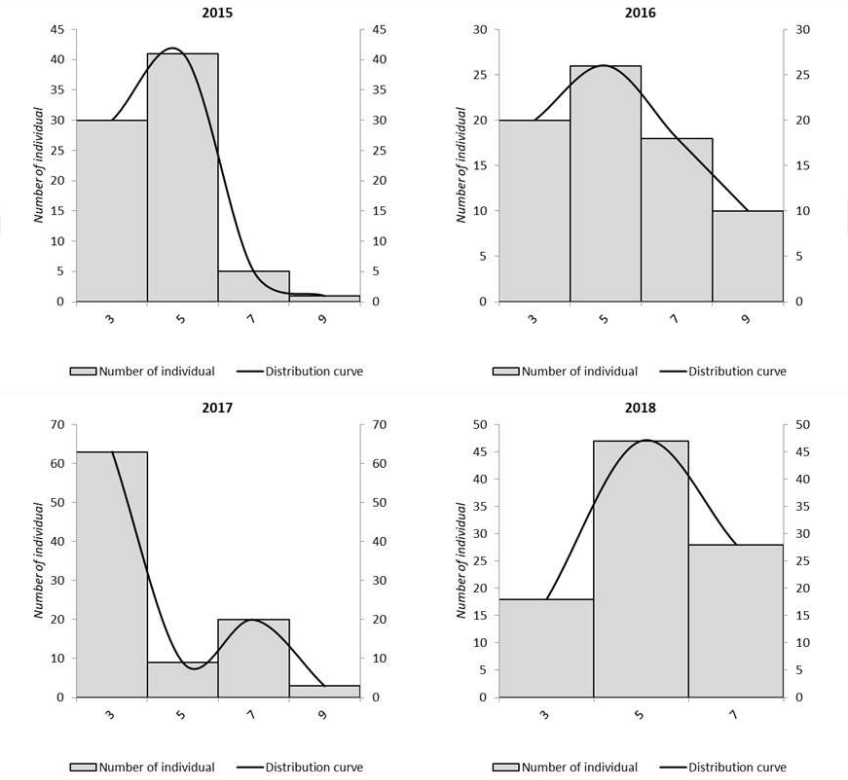

Figure 4.36. Frequency distributions of Kernel Color Intensity (KCI) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.21. Shriveling of Kernel (SoK)

Shriveling of Kernel (SoK) values in 'Gülcan-2 x Lauranne' population varied from slightly wrinkled (1), intermediate (5) and wrinkled (7) in each year. The SoK value of cv. Gülcan-2 cultivar scored as intermediate in year 2015, 2017 and 2018, while scored as slightly wrinkled in 2016. Lauranne was scored as slightly wrinkled in all four growing seasons. The standard deviation of SoK in 2015, 2016, 2017 and 2018 were 2.2, 2.0, 1.7 and 1.5, respectively (Table 4.14). The distribution of SoK in 2015 and 2016 assessed as normal distribution, while SoK showed skewed left distribution in 2017 and 2018 (Table 4.14 and Figure 4.37).

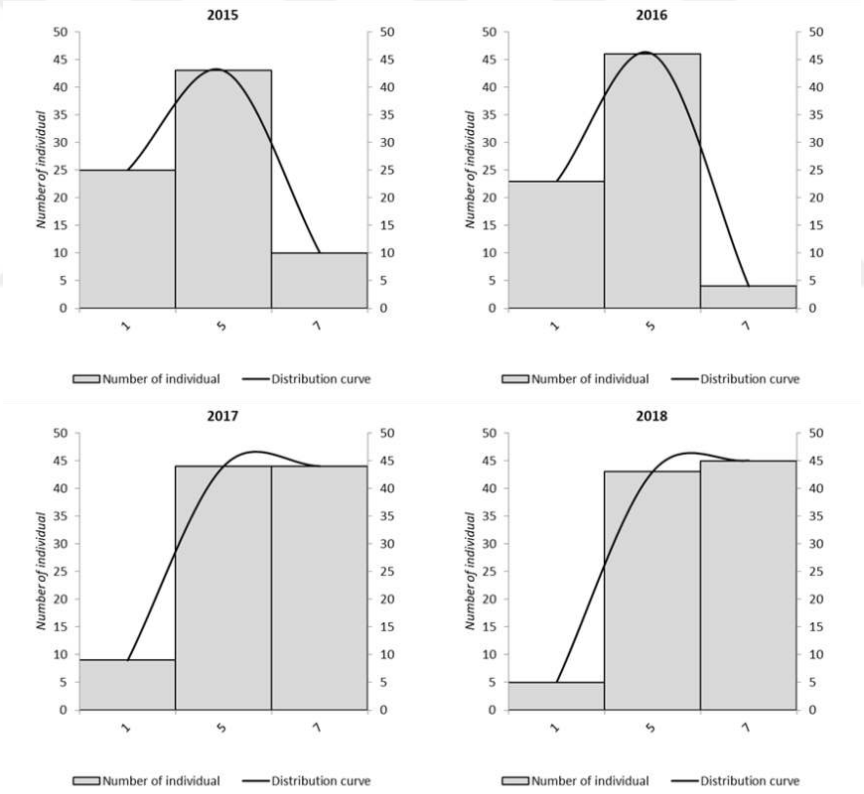

Figure 4.37. Frequency distributions of Shriveling of kernel (SoK) value in 'Gülcan-2 x Lauranne' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.22. Shell L (SL)

The Shell L (SL) value of the parents and F1 population of 'Guara x Nurlu' were measured. The SL value of cv. Guara was 48.6, 44.3, 65.2 and 54.7 in 2015, 2016, 2017 and 2018, respectively, whereas it was 40.6, 53.5, 42.5 and 52.9 in Nurlu. The ranges of SL values varied from 35.4 to 55.2, 26.6 to 54.1, 38.4 to 68.9 and 43.8 to 57.6 in 2015, 2016, 2017 and 2018, respectively. The average SL in 2015, 2016, 2017 and 2018 were 45.0, 43.3, 57.6 and 49.9, respectively. The standard deviation of SL in 2015, 2016, 2017 and 2018 were 4.5, 4.6, 5.3 and 2.5, respectively (Table 4.15). The frequency distribution of SL in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure 4.38).

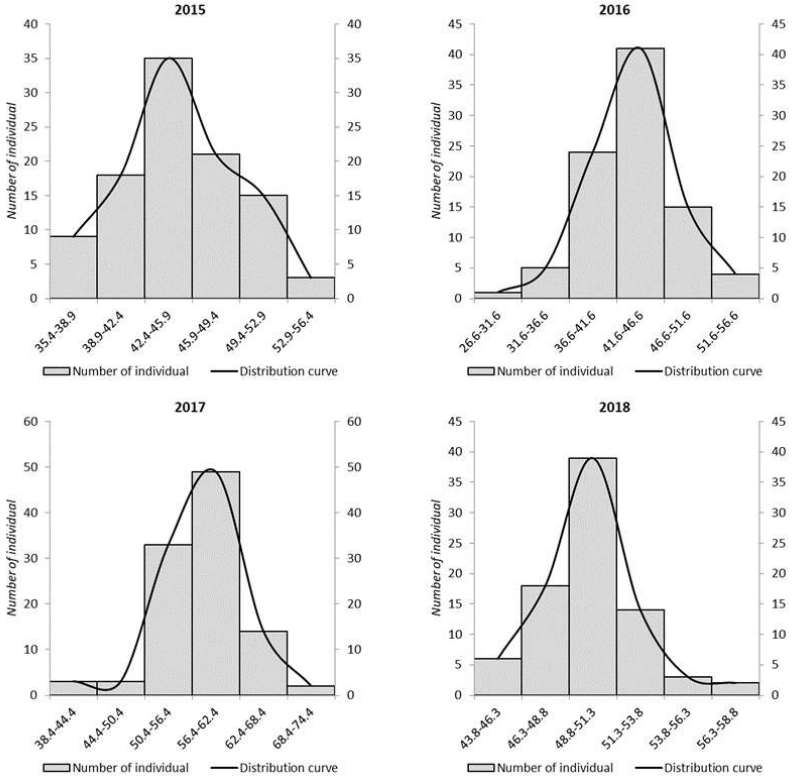

Figure 4.38. Frequency distributions of Shell L (SL) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.23. Shell a (Sa)

The Shell a (Sa) value of the parents and F1 population of 'Guara x Nurlu' were measured. The Sa value of cv. Guara cultivar was 10.9, 11.0, 8.3 and 13.5 in 2015, 2016, 2017 and 2018, respectively, whereas it was 9.8, 11.7, 10.9 and 12.2 in Nurlu. The ranges of Sa values varied from 6.3 to 15.9, 7.9 to 13.9, 5.6 to 12.8 and 10.0 to 15.0 in 2015, 2016, 2017 and 2018, respectively. The average Sa in 2015, 2016, 2017 and 2018 were 10.6, 11.4, 8.7 and 12.4, respectively. The standard deviation of in 2015, 2016, 2017 and 2018 were 1.6, 1.2, 1.3 and 1.0, respectively (Table 4.15). The frequency distribution of Sa in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure 4.39).

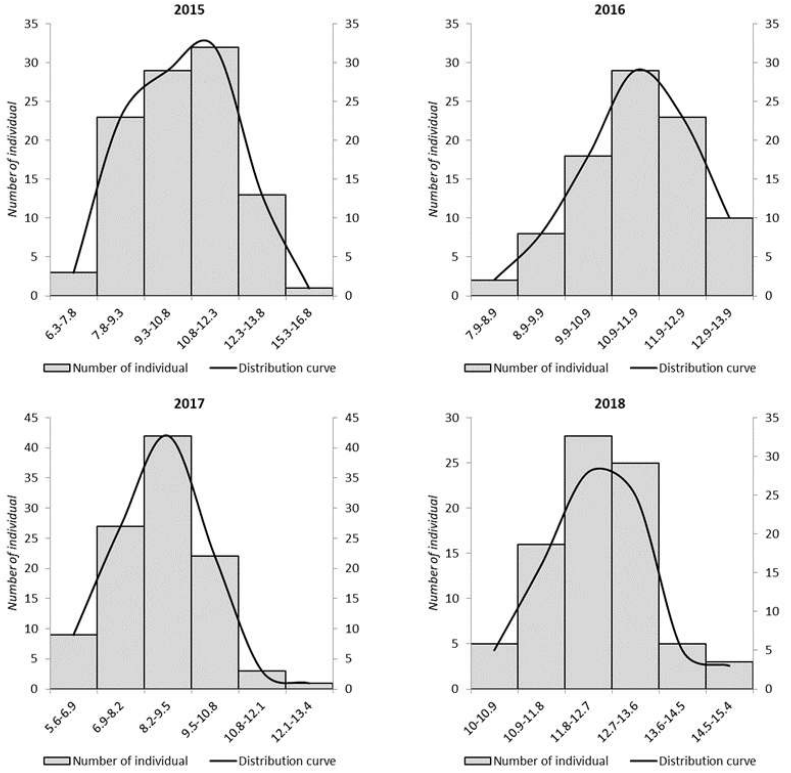

Figure 4.39. Frequency distributions of Shell a (Sa) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.24. Shell b (Sb)

The Shell b (Sb) value of the parents and F1 population of 'Guara x Nurlu' were measured. The Sb value of cv. Guara was 27.2, 22.5, 28.3 and 28.4 in 2015, 2016, 2017 and 2018, respectively, whereas it was 22.7, 27.9, 23.6 and 28.1 in Nurlu. The ranges of Sb values varied from 12.9 to 38.9, 15.2 to 29.3, 22.8 to 35.3 and 23.3 to 32.2 in 2015, 2016, 2017 and 2018, respectively. The average Sb in 2015, 2016, 2017 and 2018 were 25.2, 24.3, 27.5 and 27.6, respectively. The standard deviation of Sb in 2015, 2016, 2017 and 2018 were 4.0, 3.0, 2.3 and 1.9, respectively (Table 4.15). The frequency distribution of Sb in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure 4.40).

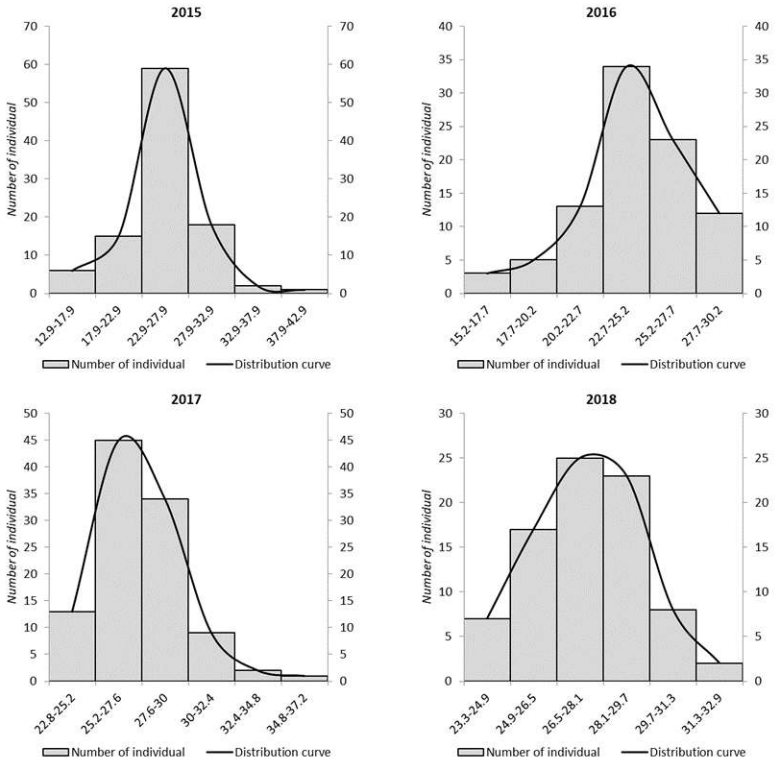

Figure 4.40. Frequency distributions of Shell b (Sb) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.25. Kernel L (KL)

The Kernel L (KL) value of the parents and F1 population of 'Guara x Nurlu' were measured. The KL value of cv. Guara was 35.5, 35.3, 51.4 and 43.9 in 2015, 2016, 2017 and 2018, respectively, whereas it was 41.4, 39.1, 40.3 and 47.3 in Nurlu. The ranges of KL values varied from 29.3 to 48.9, 28.9 to 43.3, 30.5 to 64.5 and 40.0 to 58.7 in 2015, 2016, 2017 and 2018, respectively. The average KL in 2015, 2016, 2017 and 2018 were 37.5, 35.7, 49.9 and 45.6, respectively. The standard deviation of KL in 2015, 2016, 2017 and 2018 were 3.9, 2.9, 6.1 and 1.7, respectively (Table 4.15). The frequency distribution of KL in 2015, 2016, 2017 and 2018 showed a good fit to normal distribution (Table 4.15 and Figure 4.41).

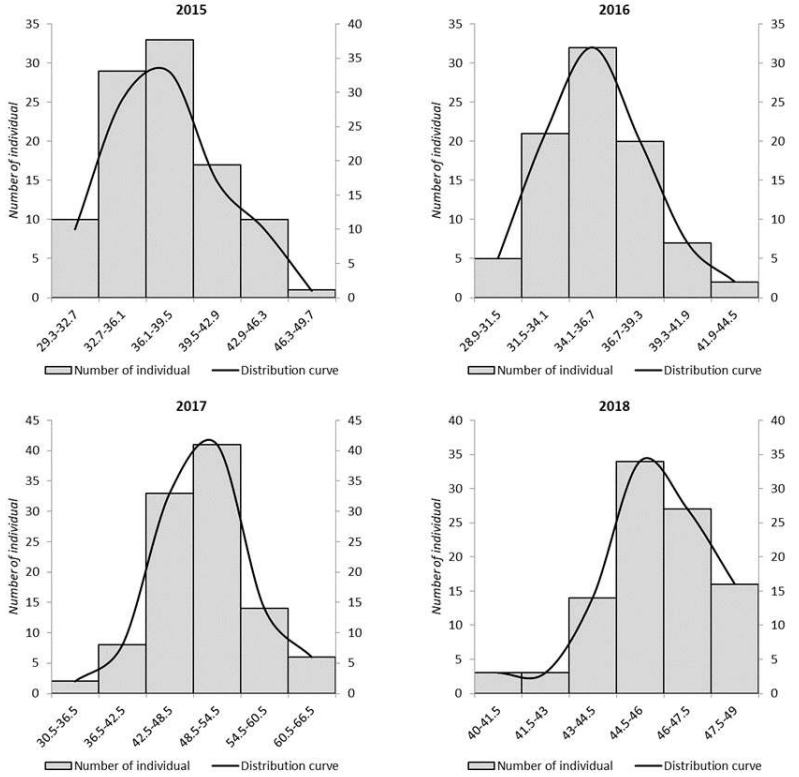

Figure 4.41. Frequency distributions of Kernel L (KL) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.26. Kernel a (Ka)

The Kernel a (Ka) value of the parents and F1 population of 'Guara x Nurlu' were measured. The Ka value of cv. Guara was 16.0, 11.9, 16.1 and 16.9 in 2015, 2016, 2017 and 2018, respectively, whereas it was 16.7, 16.1, 16.3 and 17.4 in Nurlu. The ranges of Ka values varied from 10.2 to 19.6, 12.2 to 18.5, 11.3 to 17.7 and 14.8 to 20.1 in 2015, 2016, 2017 and 2018, respectively. The average Ka in 2015, 2016, 2017 and 2018 were 15.2, 15.4, 15.0 and 16.7, respectively. The standard deviation of Ka in 2015, 2016, 2017 and 2018 were 1.6, 1.3, 1.2 and 1.0, respectively (Table 4.15). The frequency distribution of Ka in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure 4.42).

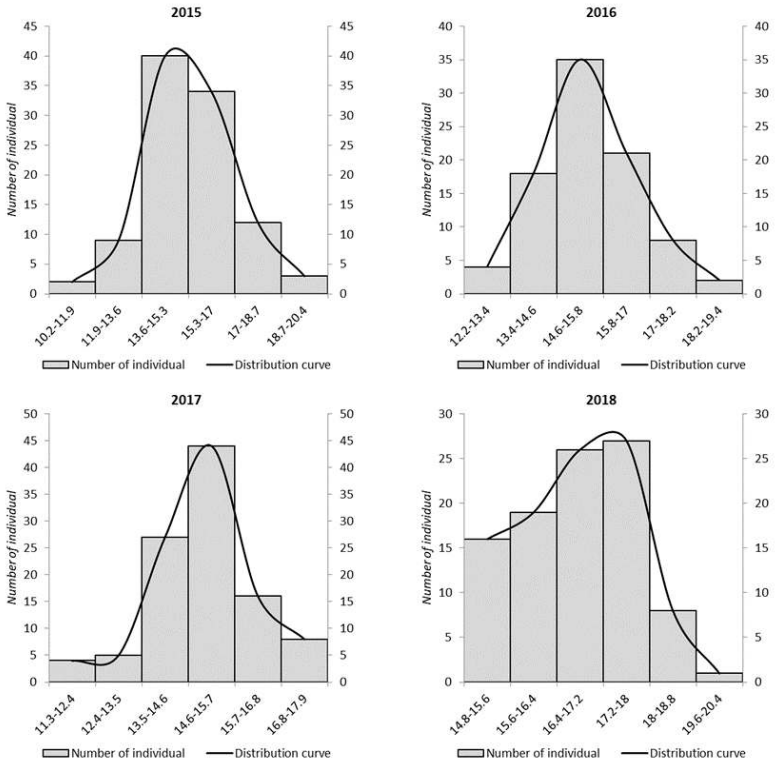

Figure 4.42. Frequency distributions of Kernel a (Ka) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.27. Kernel b (Kb)

The Kernel b (Kb) value of the parents and F1 population of 'Guara x Nurlu' were measured. The Kb value of cv. Guara was 31.0, 21.5, 39.1 and 27.3 in 2015, 2016, 2017 and 2018, respectively, whereas it was 32.3, 28.5, 29.7 and 30.7 in Nurlu. The ranges of Kb values varied from 18.8 to 45.8, 20.1 to 34.5, 24.7 to 47.8 and 22.8 to 33.9 in 2015, 2016, 2017 and 2018, respectively. The average Kb in 2015, 2016, 2017 and 2018 were 28.7, 26.5, 34.8 and 27.6, respectively. The standard deviation of Kb in 2015, 2016, 2017 and 2018 were 4.0, 2.8, 4.9 and 2.4, respectively (Table 4.15). The frequency distribution of Kb in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure.4.43).

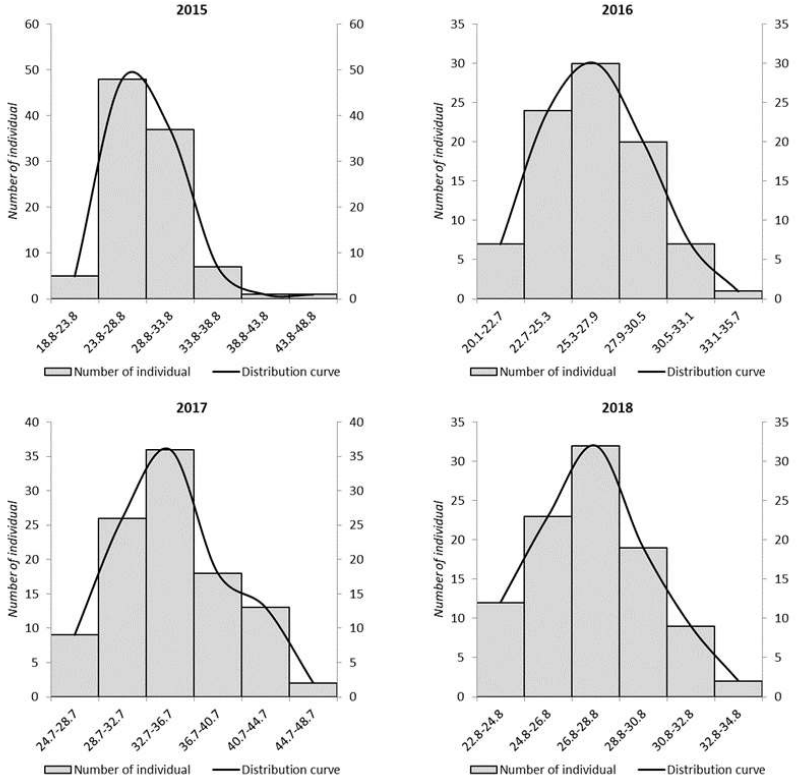

Figure 4.43. Frequency distributions of Kernel b (Kb) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.28. Nut Length (NLh)

The Nut Length (NLh) value of the parents and F1 population of 'Guara x Nurlu' were measured. The NLh value of cv. Guara was 34.4 cm, 42.9 cm, 31.4 cm and 36.2 cm in 2015, 2016, 2017 and 2018, respectively, whereas it was 31.1 cm, 40.3 cm, 35.0 cm and 34.9 cm in Nurlu. The ranges of NLh values varied from 30.4 to 44.6 cm, 29.7 to 41.4 cm, 27.2 to 41.2 cm and 29.8 to 45.0 cm in 2015, 2016, 2017 and 2018, respectively. The average NLh in 2015, 2016, 2017 and 2018 were 36.2 cm, 35.9 cm, 33.4 cm and 35.8 cm, respectively. The standard deviation of NLh in 2015, 2016, 2017 and 2018 were 2.8, 2.7, 2.5 and 2.8, respectively (Table 4.15). The frequency distribution of NLh in 2015, 2016, 2017 and 2018 showed a good fit to normal distribution (Table 4.15 and Figure 4.44).

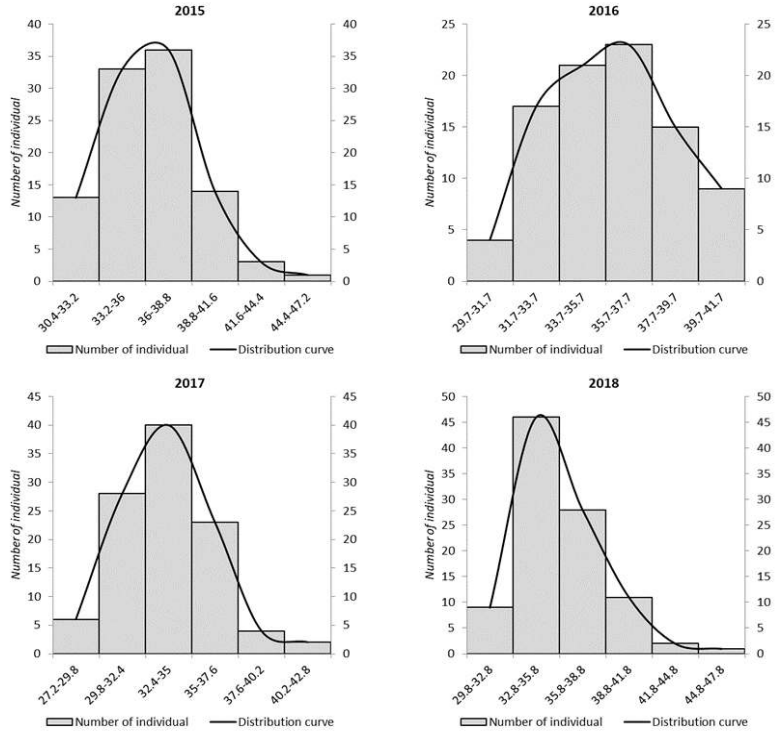

Figure 4.44. Frequency distributions of Nut Length (NLh) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.29. Nut Width (NW)

The Nut Width (NW) value of the parents and F1 population of 'Guara x Nurlu' were measured. The NW value of cv. Guara was 22.0 cm, 27.9 cm, 21.6 cm and 23.3 cm in 2015, 2016, 2017 and 2018, respectively, whereas it was 21.3 cm, 25.7 cm, 20.9 cm and 22.3 cm in Nurlu. The ranges of NW values varied from 17.7 to 27.4 cm, 17.5 to 27.9 cm, 13.7 to 25.1 cm and 17.8 to 29.9 cm in 2015, 2016, 2017 and 2018, respectively. The average NW in 2015, 2016, 2017 and 2018 were 22.3 cm, 22.4 cm, 20.6 cm and 22.4 cm, respectively. The standard deviation of NW in 2015, 2016, 2017 and 2018 were 2.1, 2.3, 2.6 and 2.0, respectively (Table 4.15). The frequency distribution of NW in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure.4.45).

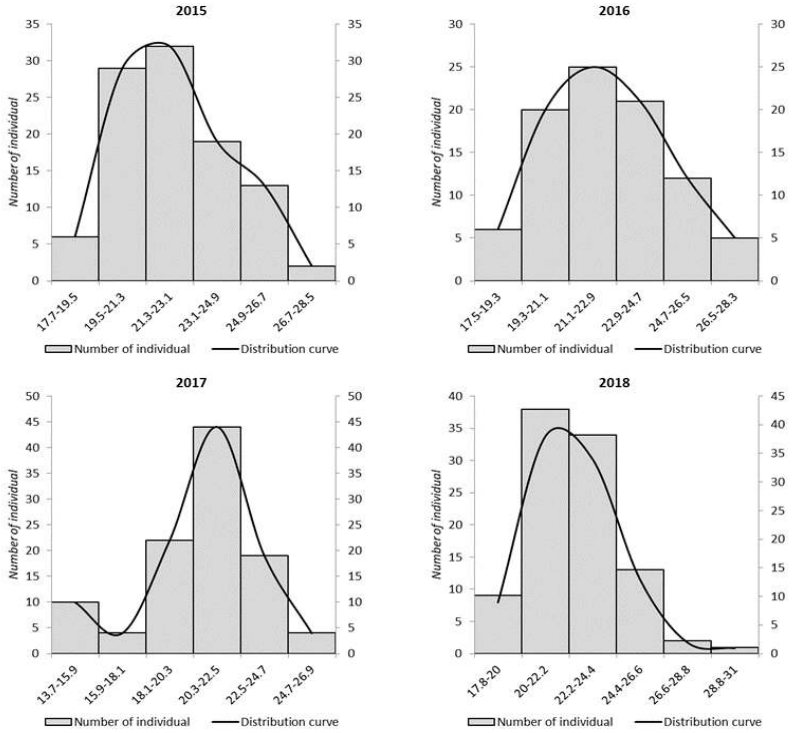

Figure 4.45. Frequency distributions of Nut Width (NW) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.30. Nut Thickness (NT)

The Nut Thickness (NT) value of the parents and F1 population of 'Guara x Nurlu' were measured. The NT value of cv. Guara was 15.0 cm, 18.8 cm, 14.2 cm and 16.2 cm in 2015, 2016, 2017 and 2018, respectively, whereas it was 13.9 cm, 17.9 cm, 15.8 cm and 16.1 cm in Nurlu. The ranges of NT values varied from 13.1 to 20.4 cm, 11.5 to 19.9 cm, 12.9 to 22.4 cm and 12.9 to 20.8 cm in 2015, 2016, 2017 and 2018, respectively. The average NT in 2015, 2016, 2017 and 2018 were 16.2 cm, 15.3 cm, 16.0 cm and 16.0 cm, respectively. The standard deviation of NT in 2015, 2016, 2017 and 2018 were 1.4, 1.4, 1.9 and 1.3, respectively (Table 4.15). The frequency distribution of NT in 2015, 2016, 2017 and 2018 showed a good fit to normal distribution (Table 4.15 and Figure 4.46).

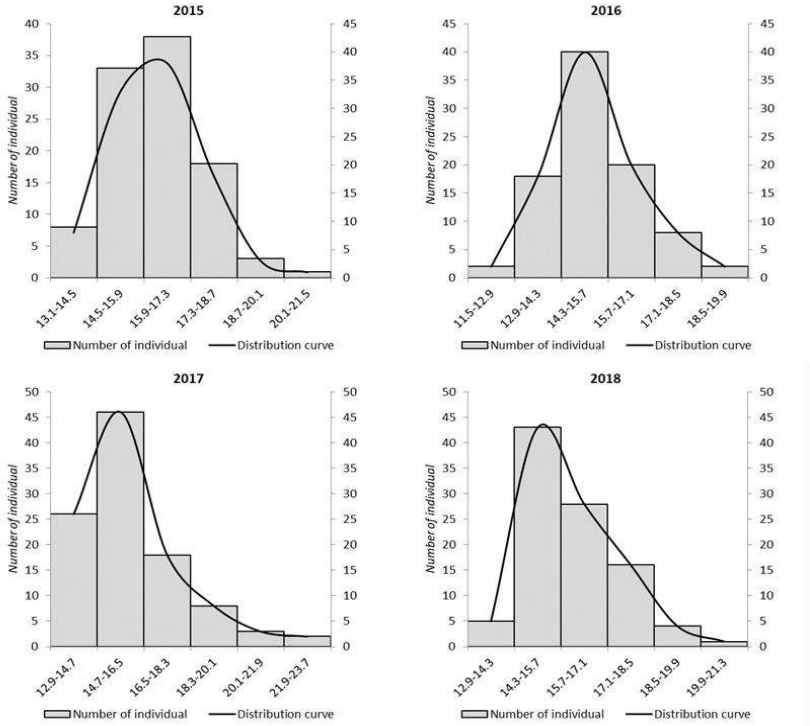

Figure 4.46. Frequency distributions of Nut Thickness (NT) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.31. Nut Weight (NWt)

The Nut Weight (NWt) value of the parents and F1 population of 'Guara x Nurlu' were measured. The NWt value of cv. Guara was 2.9 g, 6.4 g, 2.4 g and 4.4 g in 2015, 2016, 2017 and 2018, respectively, whereas it was 3.6 g, 6.2 g, 4.9 g and 5.8 g in Nurlu. The ranges of NWt values varied from 2.0 to 5.5 g, 2.8 to 7.7 g, 2.0 to 5.6 g and 2.7 to 9.5 g in 2015, 2016, 2017 and 2018, respectively. The average NWt in 2015, 2016, 2017 and 2018 were 3.4 g, 4.8 g, 3.3 g and 4.5 g, respectively (Table 4.15). The frequency distribution of NWt in 2015, 2016, 2017 and 2018 showed a good fit to normal distribution in all four years (Table 4.15 and Figure 4.47).

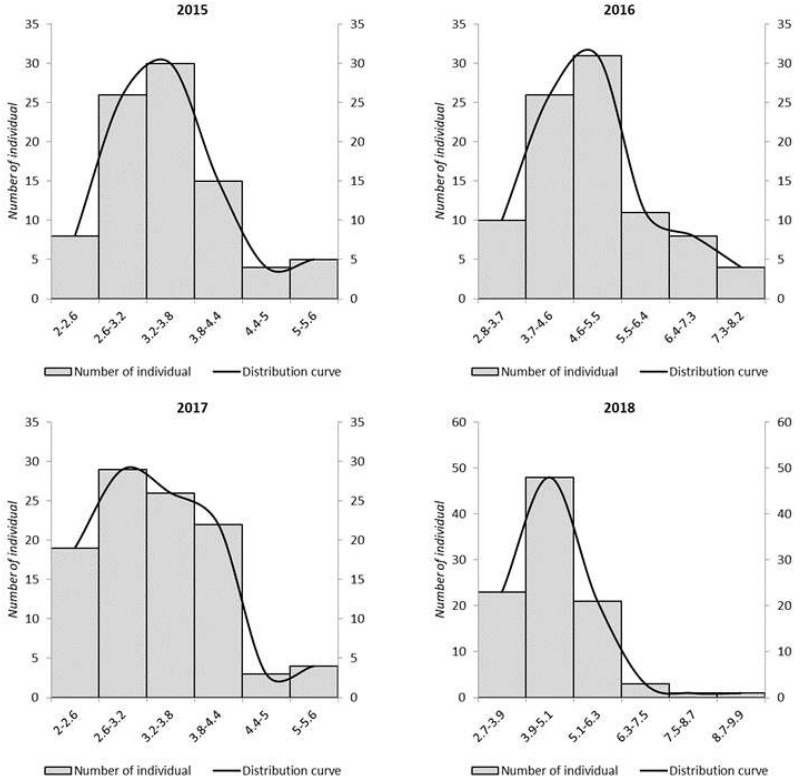

Figure 4.47. Frequency distributions of Nut Weight (NWt) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.32. Kernel Length (KLh)

The Kernel Length (KLh) value of the parents and F1 population of 'Guara x Nurlu' were measured. The KLh value of cv. Guara was 23.1 cm, 26.9 cm, 22.8 cm and 26.3cm in 2015, 2016, 2017 and 2018, respectively, whereas it was 24.6 cm, 29.5 cm, 27.1 cm and 27.7cm in Nurlu. The ranges of KLh values varied from 20.3 to 31.5 cm, 21.7 to 29.8 cm, 19.9 to 30.0 cm and 22.4 to 31.0 cm in 2015, 2016, 2017 and 2018, respectively. The average KLh in 2015, 2016, 2017 and 2018 were 24.7 cm, 25.3 cm, 24.5 cm and 26.1 cm, respectively. The standard deviation of KLh in 2015, 2016, 2017 and 2018 were 2.1, 1.8, 1.8 and 1.8, respectively (Table 4.15). The frequency distribution of KLh in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure 4.48).

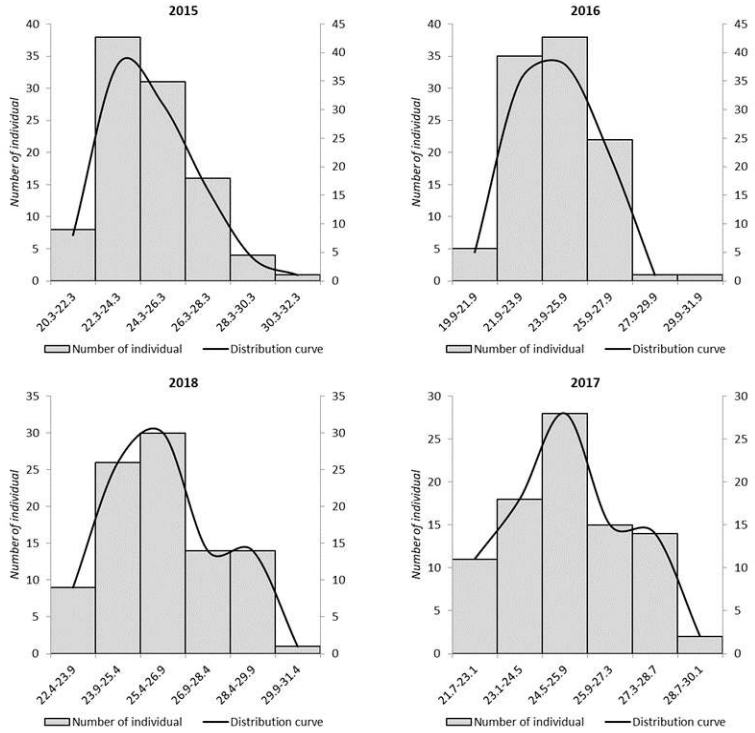

Figure 4.48. Frequency distributions of Kernel Length (KLh) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.33. Kernel Width (KW)

The Kernel Width (KW) value of the parents and F1 population of 'Guara x Nurlu' were measured. The KW value of cv. Guara was 12.8 cm, 14.3 cm, 12.8 cm and 14.2 cm in 2015, 2016, 2017 and 2018, respectively, whereas it was 10.9 cm, 15.6 cm, 12.4 cm and 14.2 cm in Nurlu. The ranges of KW values varied from 9.8 to 15.6 cm, 9.7 to 15.7 cm, 10.1 to 15.2 cm and 11.1 to 18.0 cm in 2015, 2016, 2017 and 2018, respectively. The average KW in 2015, 2016, 2017 and 2018 were ranged from 12.3 cm, 12.3 cm, 12.4 cm and 13.5 cm, respectively. The standard deviation of KW in 2015, 2016, 2017 and 2018 were 1.2, 1.2, 1.1 and 1.2, respectively (Table 4.15). The frequency distribution of KW in 2015, 2016, 2017 and 2018 showed a good fit to normal distribution (Table 4.15 and Figure 4.49).

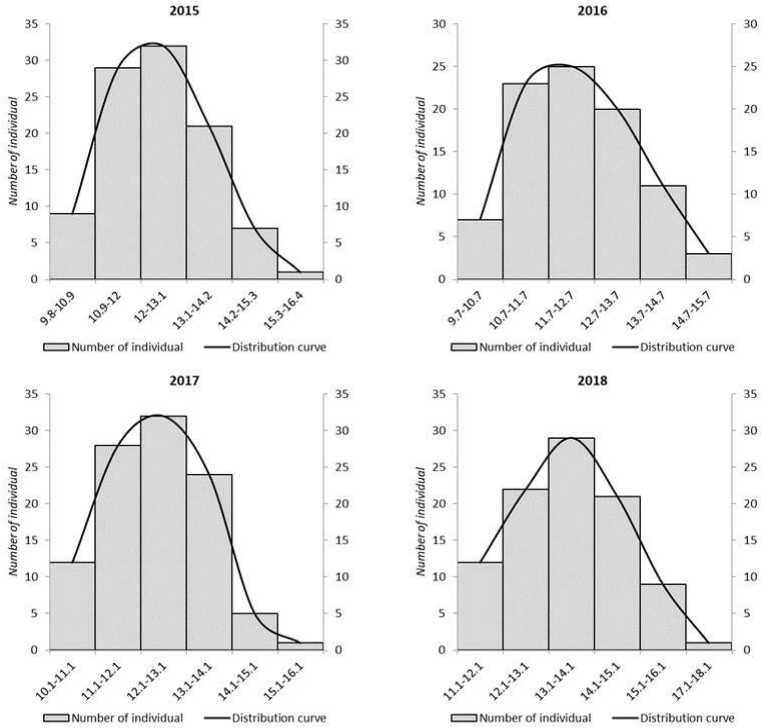

Figure 4.49. Frequency distributions of Kernel Width (KW) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.34. Kernel Thickness (KT)

The Kernel Thickness (KT) value of the parents and F1 population of 'Guara x Nurlu' were measured. The KT value of cv. Guara was 5.5 cm, 7.5 cm, 6.3 cm and 6.5 cm in 2015, 2016, 2017 and 2018, respectively, whereas it was 7.2 cm, 7.8 cm, 7.5 cm and 7.5 cm in Nurlu. The ranges of KT values varied from 3.8 to 7.3 cm, 5.4 to 7.9 cm, 5.2 to 8.2 cm and 4.5 to 8.2 cm in 2015, 2016, 2017 and 2018, respectively. The average KT in 2015, 2016, 2017 and 2018 were 5.6 cm, 6.6 cm, 6.5 cm and 6.2 cm, respectively. The standard deviation of KT in 2015, 2016, 2017 and 2018 were 0.7, 0.6, 0.7 and 0.7, respectively (Table 4.15). The frequency distribution of KT in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure 4.50).

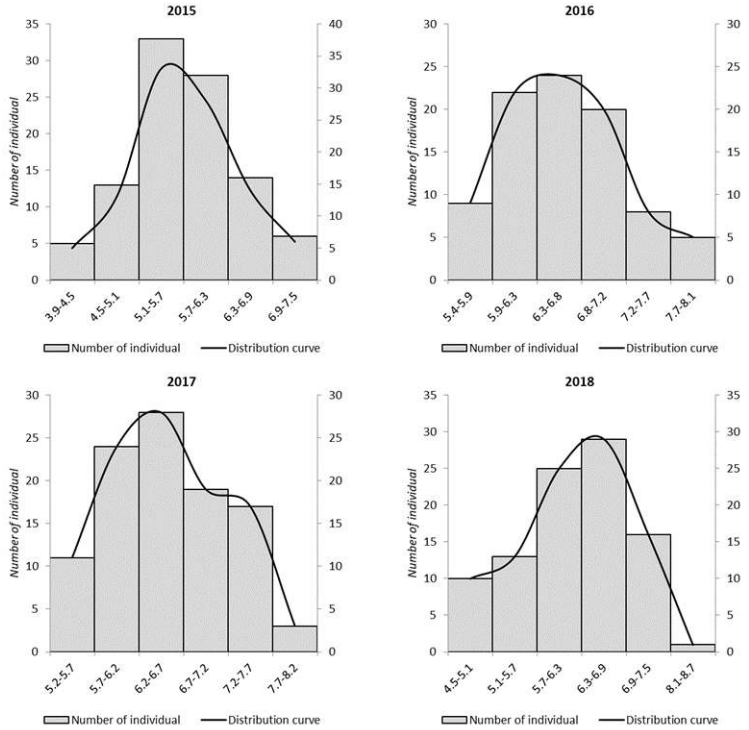

Figure 4.50. Frequency distributions of kernel thickness (KT) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.35. Kernel Weight (KWt)

The Kernel Weight (KWt) value of the parents and F1 population of 'Guara x Nurlu' were measured. The KWt value of cv. Guara was 0.9 g, 1.2 g, 0.9 g and 1.1 g in 2015, 2016, 2017 and 2018, respectively, whereas it was 0.9 g, 1.3 g, 1.2 g and 1.4 g in Nurlu. The ranges of KWt values varied from 0.5 to 1.7 g, 0.7 to 1.5 g, 0.6 to 1.5 g and 0.7 to 2.0 g in 2015, 2016, 2017 and 2018, respectively. The average KWt in 2015, 2016, 2017 and 2018 were 0.9 g, 1.1 g, 0.9 g and 1.1 g, respectively (Table 4.15). The frequency distribution of KWt in 2015 and 2017 showed a good fit to normal distribution, while showed a bimodal distribution in 2016 and 2018 (Table 4.15 and Figure 4.51).

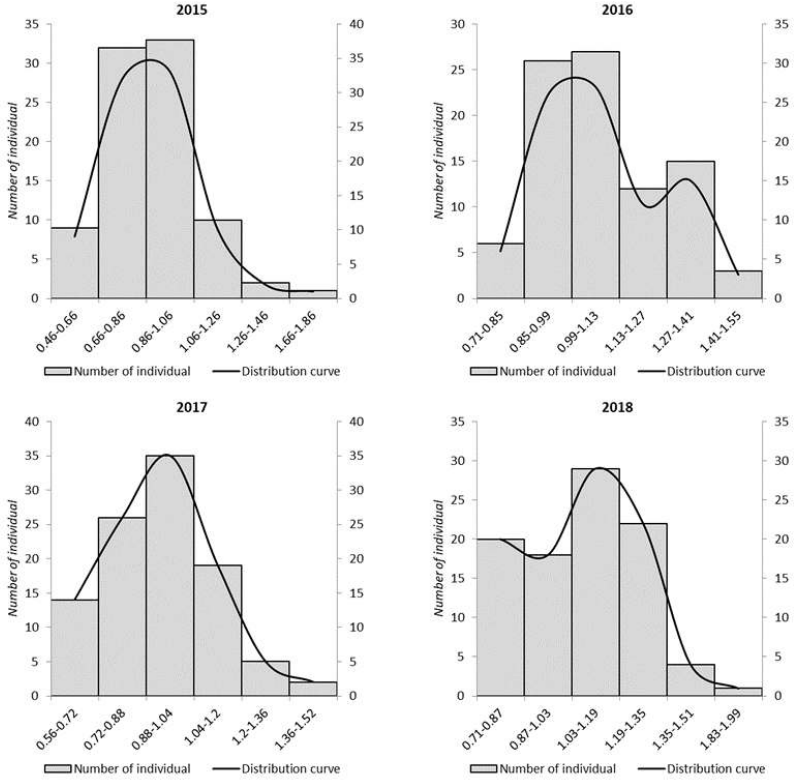

Figure 4.51. Frequency distributions of Kernel Weight (KWt) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.36. Kernel Nut Ratio (KNR)

The Kernel Nut Ratio (KNR) value of the parents and F1 population of 'Guara x Nurlu' were measured. The KNR value of cv. Guara was 30.9%, 24.8%, 35.3% and 25.7% in 2015, 2016, 2017 and 2018, respectively, whereas it was 25.6%, 20.9%, 24.6% and 24.3% in Nurlu. The ranges of KNR values varied from 13.9 to 33.1%, 15.6 to 31.3%, 17.7 to 43.4% and 14.5 to 36.0% in 2015, 2016, 2017 and 2018, respectively. The average KNR in 2015, 2016, 2017 and 2018 were 25.8%, 22.7%, 29.0% and 24.1%, respectively. The standard deviation of KNR in 2015, 2016, 2017 and 2018 were 4.0, 3.2, 4.7 and 4.4, respectively (Table 4.15). The frequency distribution of KNR in 2015, 2016, 2017 and 2018 had a good fit to normal distribution (Table 4.15 and Figure 4.52).

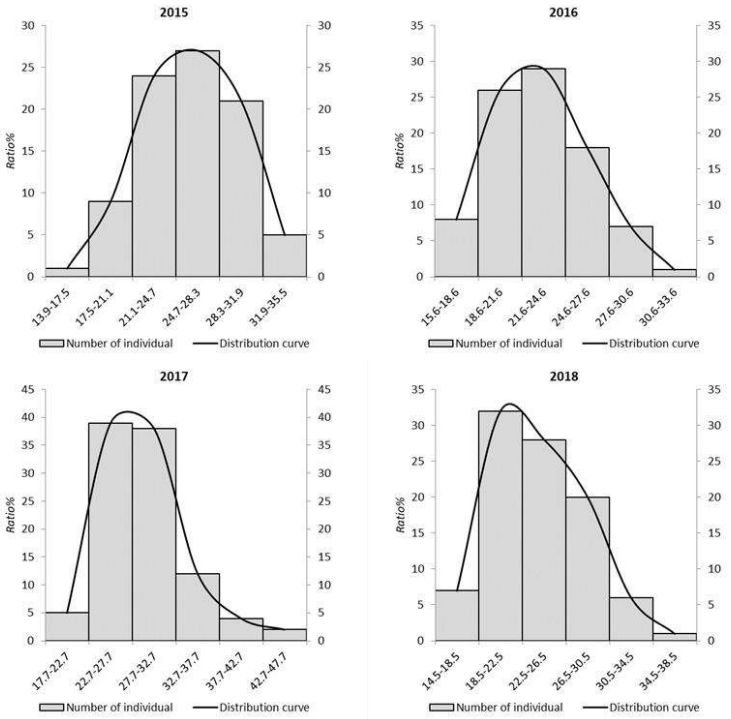

Figure 4.52. Frequency distributions of Kernel Nut Ratio (KNR) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.37. Double Kernel Ratio (DKR)

The Double Kernel Ratio (DKR) value of the parents and F1 population of 'Guara x Nurlu' were measured. The DKR value of cv. Guara was 20.0%, 36.7%, 16.7% and 66.7% in 2015, 2016, 2017 and 2018, respectively, whereas it was 0%, 0%, 13.3% and 0% in Nurlu. The ranges of DKR values varied from 0 to 100% in 2015, 2016, 2017 and 2018. The average DKR in 2015, 2016, 2017 and 2018 were 32.9%, 10.3%, 22.7% and 41.8%, respectively (Table 4.15). The frequency distribution of KNR in 2015, 2016 and 2017 had a good fit to skewed right distribution, while showed a bimodal distribution in 2018 (Table 4.15 and Figure 4.53).

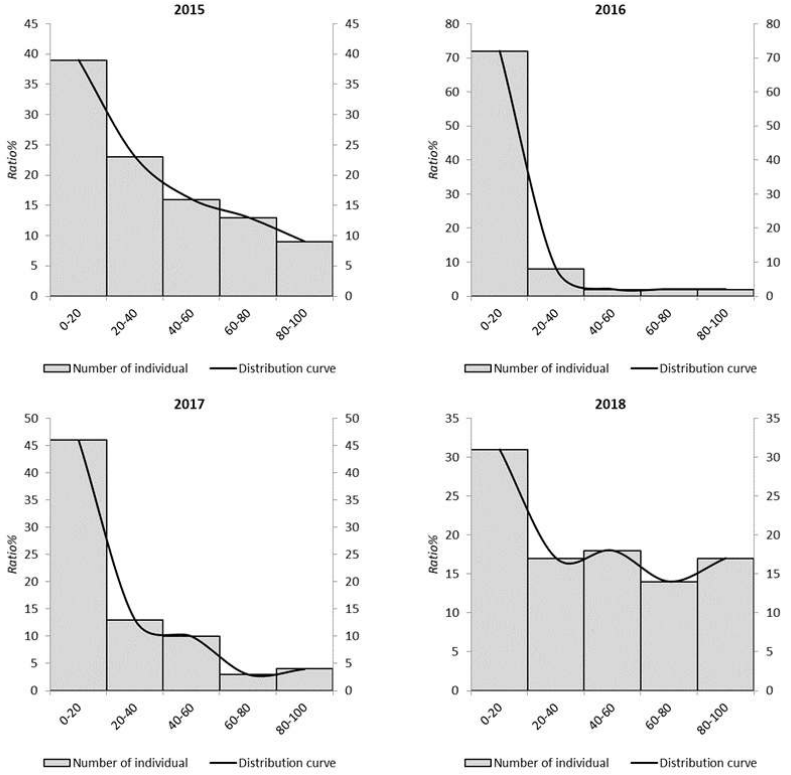

Figure 4.53. Frequency distributions of Double Kernel Ratio (DKR) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.38. Softness of Shell (SoS)

Softness of Shell (SoS) values in 'Guara x Nurlu' population varied from extremely hard (1), hard (3), intermediate (5), soft (7) and paper (9) in each year. The SoS value of cv. Guara scored as intermediate in 2015, 2017 and 2018 years and scored as hard in 2018. Nurlu was scored as hard in all four years. The standard deviation of SoS in 2015, 2016, 2017 and 2018 were 2.0, 1.4, 2.2 and 1.7, respectively (Table 4.15). The distribution of SoS in 2015, 2016 and 2018 assessed as normal distribution, while SosS value showed bimodal distribution in 2017 (Table 4.15 and Figure 4.54).

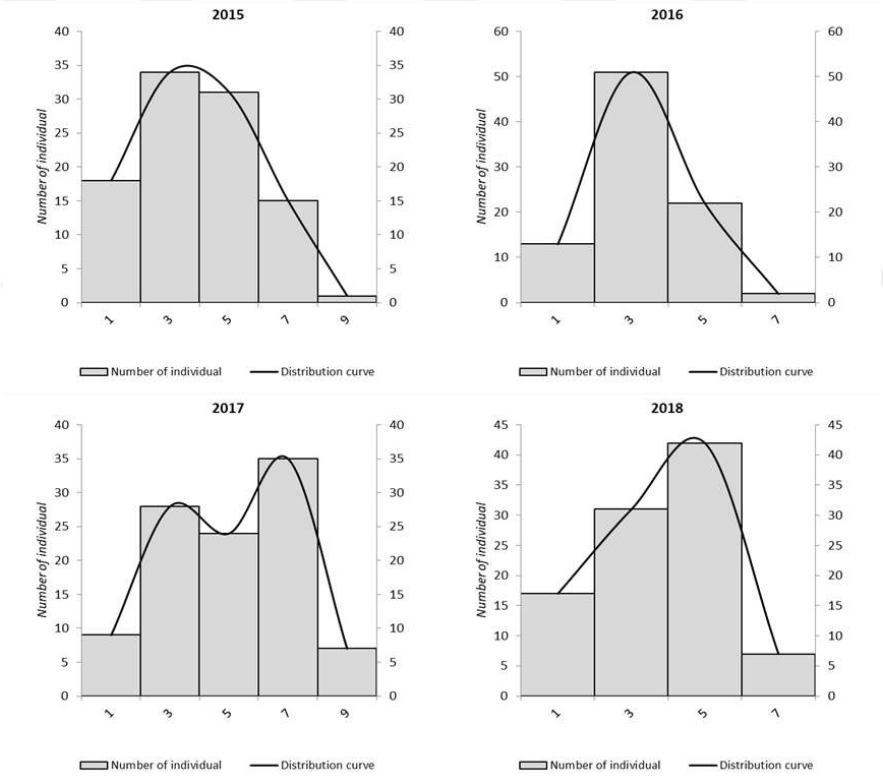

Figure 4.54. Frequency distributions of Softness of Shell (SoS) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.39. Nut Shape (NS)

Nut Shape (NS) values in 'Guara x Nurlu' population varied from round (1), ovate (3), oblong (5), cordate (7) and extremely narrow (9) in each year. The NS value of cv. Guara scored as oblong in all four growing seasons. Nurlu was scored as cordate in 2017 and 2018, and scored as extremely narrow in 2015 and 2016. The standard deviation of NS in 2015, 2016, 2017 and 2018 were 1.5, 1.6, 2.6 and 1.7, respectively (Table 4.15). The distribution of NS in 2015, 2016 and 2018 assessed as normal distribution, while NS value showed random distribution in 2017 (Table 4.15 and Figure 4.55).

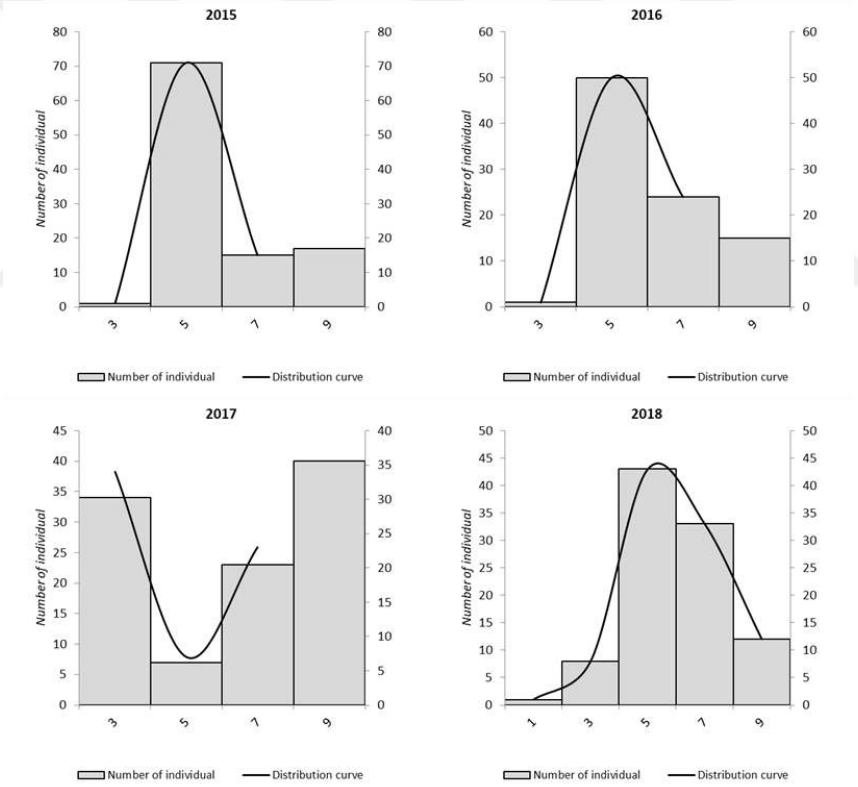

Figure 4.55. Frequency distributions of Nut Shape (NS) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.40. Shell Color Intensity (SCI)

Shell Color Intensity (SCI) values in 'Guara x Nurlu' population varied from extremely light (1), light (3), intermediate (5), dark (7) and extremely dark (9) in each year. The SCI value of cv. Guara scored as dark in year 2016 and 2018, and scored as extremely dark in year 2015 and 2017. Nurlu was also scored as dark in 2015, 2016 and 2018 years and scored as intermediate in 2017. The standard deviation of SCI in 2015, 2016, 2017 and 2018 were 1.7, 2.0, 1.6 and 2.1, respectively (Table 4.15). The distribution of SCI in 2015, 2016, 2017 and 2018 showed a normal distribution in all four years (Table 4.15 and Figure 4.56).

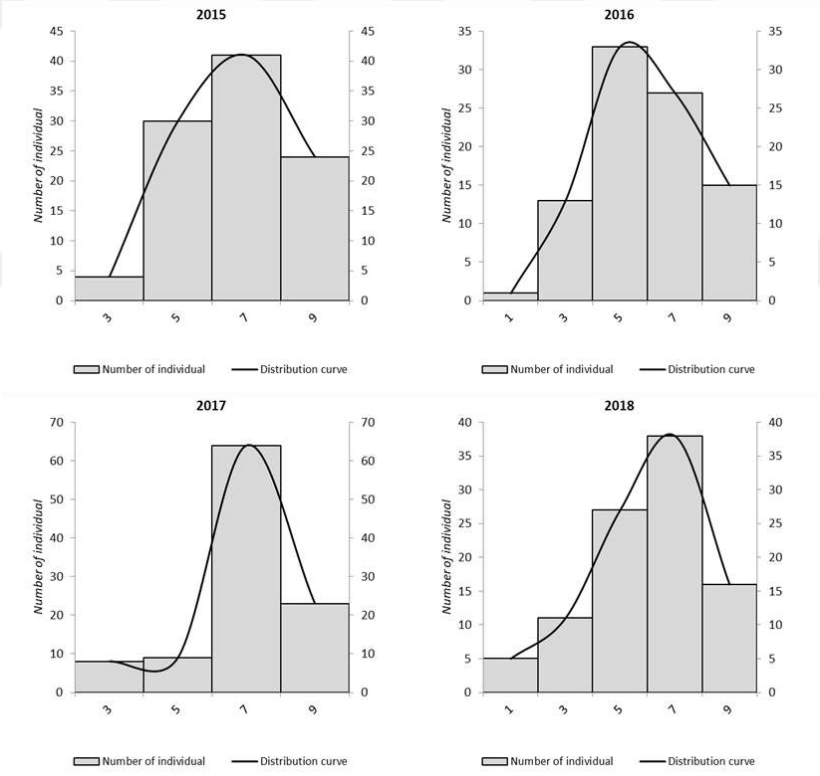

Figure 4.56. Frequency distributions of Shell Color Intensity (SCI) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.41. Kernel Color Intensity (KCI)

Khell Color Intensity (KCI) values in 'Guara x Nurlu' population varied from extremely light (1), light (3), intermediate (5), dark (7) and extremely dark (9) in each year. The cv. Guara was scored as dark in 2015, scored as intermediate in 2016, while scored as light in 2017 and 2018. The KCI of cv. Nurlu scored as dark in all four years. The standard deviation of KCI in 2015, 2016, 2017 and 2018 were 1.6, 1.6, 2.2 and 2.1, respectively (Table 4.15). The distribution of KCI in 2015, 2016 and 2018 assessed as normal distribution, while KCI had a bimodal distribution in 2017 (Table 4.15 and Figure 4.57).

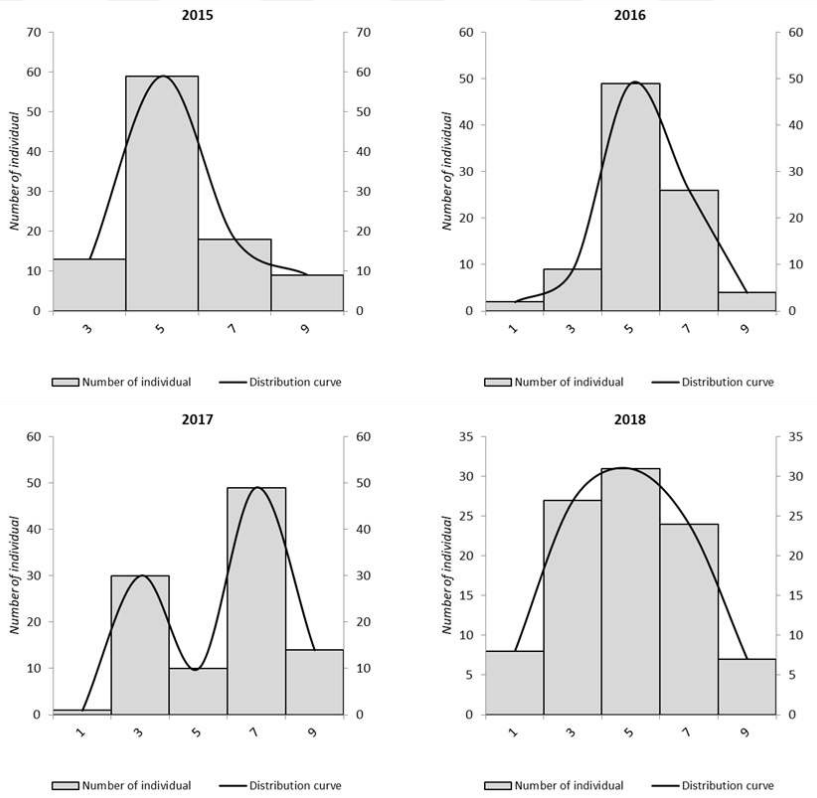

Figure 4.57. Frequency distributions of Kernel Color Intensity (KCI) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.2.42. Shriveling of Kernel (SoK)

Shriveling of Kernel (SoK) values in 'Guara x Nurlu' population varied from slightly wrinkled (1), intermediate (5) and wrinkled (7) in each year. According to the observations, the SoK values of two parents Guara and Nurlu were similar. The SoK value of cv. Guara scored as intermediate in all four years. Nurlu was also scored as intermediate in 2016, 2017 and 2018, while scored as wrinkled in 2015. The standard deviation of SoK in 2015, 2016, 2017 and 2018 were 2.1, 2.0, 1.5 and 2.4, respectively (Table 4.15). The distribution of SoK in 2015 and 2016 had a good fit to normal distribution, while SoK showed skewed left distribution in 2017 and showed skewed right distribution in 2018. (Table 4.15 and Figure 4.58).

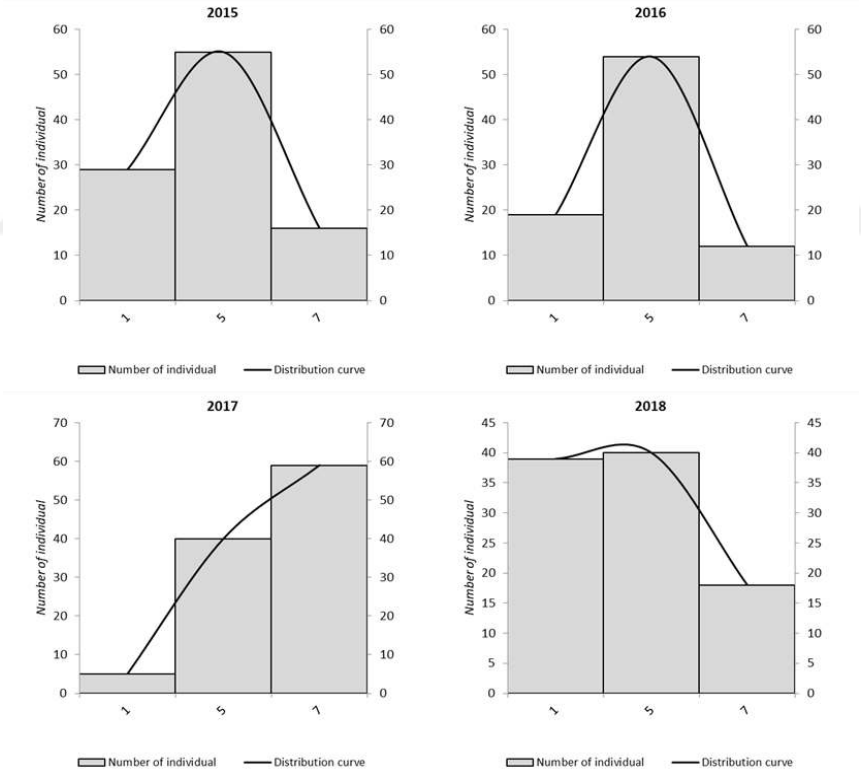

Figure 4.58. Frequency distributions of Shriveling of kernel (SoK) value in 'Guara x Nurlu' population. Phenotypic data were collected in 2015, 2016, 2017 and 2018.

4.2.3. QTL Identification for Nut and Kernel Traits in Two Almond Populations

The parental maps of 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations were used for the QTL analysis. Phenotypic data of 21 traits were collected and used for QTL analysis. The QTLs detected at least in two growing seasons were considered significant. In 'Gülcan-2 x Lauranne' population, a total of 12 and 11 QTLs related to nine traits were identified in the Gülcan-2 and in Lauranne linkage maps, respectively. In 'Guara x Nurlu' population, 15 traits were successfully mapped with 23 QTLs in the Guara genetic map, while 22 QTLs related to 14 traits were detected in the Nurlu map (Table 4.16, Table 4.17 and Table 4.18).

4.2.3.1. Shell L (SL)

In the 'Guara x Nurlu' population, one significant QTL for Shell L (SL) was mapped into the middle part of the LG1 of the Guara map in 2017 and 2018. There was no significant QTL for SL in the Nurlu map. The highest LOD scores were 3.3 and 3.0, and the highest percentages of the explained phenotypic variations were 13.8% and 16.4% in 2017 and 2018, respectively. The closest marker in LG1 was 7305131 (32.4 cM) in both years (Table 4.17, Table 4.18 and Figure 4. 60).

4.2.3.2. Shell a (Sa)

In the 'Gülcan-2 x Lauranne' population, one significant QTL for Shell a (Sa) was mapped into the upper part of the LG4 in 2017 and 2018. The highest LOD score was 3.2 in both years, and the highest percentages of the explained phenotypic variations (PV) were 14.5% and 14.7% in 2017 and 2018, respectively in cv. Gülcan-2 map. In the Lauranne map, the highest LOD score was 3.8 in 2017 with 17.4% phenotypic variation. The closest marker in LG4 was pchgms2 (5.9 cM) in both years and in the both parental maps (Table 4.16, Table 4.18 and Figure 4.59).

4.2.3.3. Kernel a (Ka)

In the 'Guara x Nurlu' population, one significant QTL for Kernel a (Ka) was mapped into the middle-upper part of the LG8. This QTL was detected in the Guara map in 2015, 2016 and 2018, while the QTL was detected in 2015 and 2018 in the Nurlu map. In cv. Guara map, the highest LOD scores were 3.2, 3.0 and 10.5, and the highest explained phenotypic variations were 13.8%, 14.8% and 39.2% in 2015, 2016 and 2018, respectively. The closest marker in LG8 was 7304874 (14.4 cM) in all three years in Guara map. In cv. Nurlu map, the highest LOD scores were 3.8 and 10.5, and the highest explained phenotypic variations were 16.3% and 39.9% in 2015 and 2018, respectively. The closest marker in LG8 was UDAp-470 at 30.2 cM in all three years (Table 4.17, Table 4.18 and Figure 4.60).

4.2.3.4. Nut Length (NLh)

In the 'Gülcan-2 x Lauranne' population, two significant QTLs were detected for Nut Length (NLh). One of the QTL was mapped into the middle part of the LG2 in 2016, 2017 and 2018 in the Gülcan-2 map and in 2016 and 2018 growing seasons in the cv. Lauranne map. The highest LOD scores were 3.5, 3.1 and 3.6, and the highest explained phenotypic variations were 17.5%, 13.7% and 17.8% in the cv. Gülcan-2 map in 2016, 2017 and 2018, respectively. In the Lauranne map, the highest LOD score was 3.6 in 2016 with 17.7% phenotypic variation. The closest markers in LG2 was UDP96-013 (38.8 cM) in the Gülcan-2 map and 7303716 at 32.4 cM in the cv. Lauranne map in all years (Table 4.16, Table 4.18 and Figure 4.59).

The other QTL in the 'Gülcan-2 x Lauranne' population for NLh was identified in the upper part of LG6 in both maps in 2015, 2017 and 2018. In the Gülcan-2 map, the highest LOD scores were 3.9, 4.3 and 4.4, and the highest explained phenotypic variations were 23.1%, 19.8% and 21.3% in 2015, 2017 and 2018, respectively. In cv. Lauranne map, the highest LOD scores were 5.1, 5.9 and 4.8, and the highest explained phenotypic variations were 23.5%, 25.3% and 21.9%

in 2015, 2017 and 2018, respectively. The closest marker in LG6 was 7301017 at 2.3 cM in the Gülcan-2 map and 7312614 (2.0 cM) in cv. Lauranne map in all three years (Table 4.16, Table 4.18 and Figure 4.59).

In the 'Guara x Nurlu' population, two significant QTLs were detected for Nut Length (NLh). One of the QTL was mapped into the upper part of the LG7 in 2015, 2017 and 2018 in both Guara and Nurlu maps. The highest LOD scores were 4.1, 3.6 and 4.4, and the highest percentages of the explained phenotypic variations were 18.8%, 17.2% and 22.6% in 2015, 2017 and 2018 in cv. Guara map, respectively. In Nurlu map, the highest LOD scores were 3.1, 3.3 and 2.9, and the highest explained phenotypic variations were 12.1%, 13.3% and 17.4% in 2015, 2017 and 2018, respectively The closest marker in LG7 was UDA-009 in the both maps in all growing seasons (Table 4.17, Table 4.18 and Figure 4.60).

The other QTL in the 'Guara x Nurlu' population for NLh was detected on the lower part of LG8 in both maps in 2015, 2017 and 2018. In the Guara map, the highest LOD scores were 3.3, 5.2 and 4.7, and the highest percentages of the explained phenotypic variations were 14.2%, 21.3% and 20.6% in 2015, 2017 and 2018, respectively. In cv. Nurlu map, the highest LOD scores were 3.3, 5.3 and 4.8, and the highest percentages of the explained phenotypic variations were 15.6%, 21.3% and 20.8% in 2015, 2017 and 2018, respectively. The closest marker in LG8 was 7298980 (43.1 cM) in the cv. Guara map and 7300235 at 55.8 cM in the Nurlu map in all three years (Table 4.17, Table 4.18 and Figure 4.60).

Fernandez I Marti et al. (2013) detected QTL related to the nut length on LG1 (4.6 LOD and 30.5% PV), LG5 (3.3 LOD and 18.9% PV), LG6 (3.8 LOD and 23.0% PV) and LG7 (2.2 LOD and 15.0% PV), Font i Forcada et al. (2015) mapped nut length in LG5 and LG6 in almond association mapping study. In this study, QTL related to nut length was mapped on LG2 (3.6 LOD and 17.8% PV) and LG6 (4.4 LOD and 21.3% PV) in 'Gülcan-2 x Lauranne' population, while mapped on LG7 (4.4 LOD and 22.6% PV), and LG8 (5.2 LOD and 21.3% PV) in 'Guara x Nurlu' population.

4.2.3.5. Nut Width (NW)

In the 'Gülcan-2 x Lauranne' population, one significant QTL for Nut Width (NW) was mapped into the middle-upper part of the LG2 in 2016, 2017 and 2018 in both maps. The highest LOD scores were 3.2, 4.4 and 4.6, and the highest percentages of the explained phenotypic variations were 15.2%, 17.5% and 20.8% in 2016, 2017 and 2018 in cv. Gülcan-2 map, respectively. In the Lauranne map, the highest LOD scores were 3.2, 4.1 and 4.6, and the highest percentages of the explained phenotypic variations were 15.2%, 17.5% and 21.0% in 2016, 2017 and 2018, respectively. The closest marker in LG2 was MA005a in the both maps in all three years (Table 4.16, Table 4.18 and Figure 4.59).

In the 'Guara x Nurlu' population, one significant QTL for Nut Width (NW) was mapped into the middle-upper part of the LG6 in the year 2015, 2016 and 2018 in the Guara map and in 2015 and 2018 in cv. Nurlu map. In the Guara map, the highest LOD scores were 4.8, 3.2 and 4.5, and the highest percentages of the explained phenotypic variations were 20.6%, 15.9% and 19.6% in 2015, 2016 and 2018, respectively. In the Nurlu map, the highest LOD scores were 4.8 and 4.5, and the highest explained phenotypic variations were 19.7% and 19.1% in 2015 and 2018, respectively. The closest marker in LG6 was CPPCT023 in the both maps in all years (Table 4.17, Table 4.18 and Figure 4.60).

Fernandez I Marti et al. (2013) detected QTL related to the nut width on LG2 (3.0 LOD and 19.6% PV) and LG3 (3.0 LOD and 19.0% PV). Font i Forcada et al. (2015) mapped nut width in LG3 in almond association mapping study. In this study, QTL related to nut width mapped on LG2 (4.6 LOD and 20.8% PV) in 'Gülcan-2 x Lauranne' population, while mapped on LG6 (4.8 LOD and 20.6% PV) in 'Guara x Nurlu' population.

4.2.3.6. Nut Thickness (NT)

In the 'Guara x Nurlu' population, one significant QTL for Nut Thickness (NT) was mapped into the middle-upper part of the LG6 in 2015 and 2018 in both

linkage maps. The highest LOD scores were 4.3 and 5.5, and the highest percentages of the explained phenotypic variations were 19.0% and 23.0% in 2015 and 2018 in cv. Guara map, respectively. In the Nurlu map, the highest LOD scores were 4.9 and 5.5, and the highest explained phenotypic variations were 20.0% and 23.0% in 2015 and 2018, respectively. The closest marker in LG6 was CPPCT023 in both years in the both linkage maps (Table 4.17, Table 4.18 and Figure 4.60).

Fernandez i Marti et al. (2013) detected the QTL related to the nut thickness on LG2 (2.3 LOD and 16.0% PV), LG3 (2.7 LOD and 21.7% PV) and LG7 (3.6 LOD and 23.0% PV). Font i Forcada et al. (2015) mapped nut thickness in LG3 in almond association mapping study. In this study, QTL related to nut thickness mapped on LG6 (5.5 LOD and 23.0% PV) in 'Guara x Nurlu' population.

4.2.3.7. Nut Weight (NWt)

In the 'Gülcan-2 x Lauranne' population, two significant QTLs were identified for Nut Weight (NWt) in the Gülcan-2 map and one QTL was detected in cv. Lauranne map. One QTL for NWt was mapped into the middle part of the LG2 in 2016, 2017 and 2018 in the Gülcan-2 map. The highest LOD scores were 3.4, 4.1 and 5.1, and the highest percentages of the explained phenotypic variations were 15.3%, 16.8% and 20.0% in 2016, 2017 and 2018 in cv. Gülcan-2 map, respectively. The closest marker in LG2 was UDP96-013 at 38.8 cM in all years in cv. Gülcan-2 map (Table 4.16, Table 4.18 and Figure 4.59).

The other QTL in the 'Gülcan-2 x Lauranne' population for NWt was detected in the lower part of LG6 in both maps during 2015 and 2018. The highest LOD scores were 3.4 and 3.7, and the highest percentages of the explained phenotypic variations were 17.0% and 17.6% in 2015 and 2018 in cv. Gülcan-2 map, respectively. In the Lauranne map, the highest LOD scores were 3.4 and 5.0, and the highest explained phenotypic variations were 16.9% and 20.0% in 2015 and 2018, respectively. The closest markers in LG5 was EPDCU4658 (41.1 cM) in the cv.

Gülcan-2 map and 7314035 at 54.7 cM in the Lauranne map in both years (Table 4.16, Table 4.18 and Figure 4.59).

In the 'Guara x Nurlu' population, two significant QTLs were detected for Nut Weight (NWt). One QTL was mapped into the lower part of the LG1 in 2017 and 2018 in both Guara and Nurlu maps. The highest LOD scores were 3.7 and 4.3, and the highest percentages of the explained phenotypic variations were 14.5% and 14.2% in 2017 and 2018 in cv. Guara map, respectively. In the Nurlu map, the highest LOD scores were 4.5 and 4.7, and the highest explained phenotypic variations were 17.4% and 15.5% in 2017 and 2018, respectively. The closest marker in LG1 was PMS67 in both years in both linkage maps (Table 4.17, Table 4.18 and Figure 4.60).

The other QTL in the 'Guara x Nurlu' population was identified in the middle part of LG6 in both maps in 2016 and 2018 growing seasons. The highest LOD scores were 4.2 and 5.4, and the highest percentages of the explained phenotypic variations were 20.3% and 23.2% in 2016 and 2018 in cv. Guara map, respectively. In the Nurlu map, the highest LOD scores were 3.8 and 5.3, and the highest explained phenotypic variations were 18.1% and 22.3% in 2016 and 2018, respectively. The closest marker in LG6 was CPPCT023 in both years in both linkage maps (Table 4.17, Table 4.18 and Figure 4.60).

Sanchez-Perez et al. (2007) mapped QTL related to nut weight on LG1 (3.3 LOD and 13.8% PV) and LG2 (8.7 LOD and 29.3% PV). Fernandez I Marti et al. (2013) also detected the QTL related to the nut weight on LG1 (2.5 LOD and 16.8% PV) and LG2 (4.9 LOD and 27.8% PV). Font i Forcada et al. (2015) failed to map this trait on the linkage map. Donoso et al. (2016) maped fruit weigh on LG6 in an interspecific almond x peach population. In this study, QTL related to nut weight mapped on LG2 (5.1 LOD and 20.0% PV) and LG5 (3.7 LOD and 17.6% PV) in 'Gülcan-2 x Lauranne' population, while mapped on LG1 (4.3 LOD and 14.2% PV) and LG6 (5.4 LOD and 23.2% PV) in 'Guara x Nurlu' population.

4.2.3.8. Kernel Length (KLh)

In the 'Gülcan-2 x Lauranne' population, one significant QTL for Kernel Length (KLh) was mapped into the upper part of the LG6 in 2015, 2017 and 2018 in both parental maps. The highest LOD scores were 4.5, 3.8 and 6.5, and the highest percentages of the explained phenotypic variations were 25.5%, 17.5% and 30.4% in 2015, 2017 and 2018 in cv. Gülcan-2 map, respectively. In the Lauranne map, the highest LOD scores were 4.7, 6.4 and 8.0, and the highest explained phenotypic variations were 27.7%, 29.4% and 31.5% in 2015, 2017 and 2018, respectively. The closest marker in LG6 was 7301017 (2.3 cM) in cv. Gülcan-2 map and 7312614 at 2.0 cM in the Lauranne map in all three years (Table 4.16, Table 4.18 and Figure 4.59).

In the 'Guara x Nurlu' population, two significant QTLs were detected for Kernel Length (KLh). One QTL was mapped into the lower part of the LG6 in 2017 and 2018 in both parental maps. The highest LOD scores were 6.4 and 8.7, and the highest percentages of the explained phenotypic variations were 25.7% and 36.8% in 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 5.9 and 8.3, and the highest explained phenotypic variations were 25.4% and 36.8% in 2017 and 2018, respectively. The closest marker in LG6 was 7298980 at 43.1 cM in the Guara map and 7300235 (55.8 cM) in cv. Nurlu map in both years (Table 4.17, Table 4.18 and Figure 4.60).

The other QTL in the 'Guara x Nurlu' population for KLh was identified in the upper middle part of LG8 in both maps in all growing seasons. The highest LOD scores were 5.0, 3.7, 4.1 and 4.1, and the highest percentages of the explained phenotypic variations were 21.4%, 18.2%, 16.8% and 18.2% in 2015, 2016, 2017 and 2018 in cv. Guara map, respectively. In the Nurlu map, the highest LOD scores were 5.0, 3.6, 4.2 and 4.3, and the highest explained phenotypic variations were 20.9%, 18.0%, 17.6% and 19.2% in 2015, 2016, 2017 and 2018, respectively. The closest marker in LG8 was MA042a in all four years in both linkage maps (Table 4.17, Table 4.18 and Figure 4.60).

Fernandez I Marti et al. (2013) detected the QTL related to the kernel length on LG1 (3.9 LOD and 27.2% PV), LG5 (3.9 LOD and 16.8% PV), LG6 (2.5 LOD and 23.6% PV) and LG7 (2.1 LOD and 16.4% PV). Font i Forcada et al. (2015) mapped kernel length in LG1, LG5 and LG6 in almond association mapping study. In this study, QTL related to kernel length mapped on LG6 (6.5 LOD and 30.4% PV) in 'Gülcan-2 x Lauranne' population, while mapped on LG6 (8.7 LOD and 36.8% PV) and LG8 (4.1 LOD and 18.2% PV) in 'Guara x Nurlu' population.

4.2.3.9. Kernel Width (KW)

In the 'Guara x Nurlu' population, two significant QTLs were detected for Kernel Width (KW). One QTL was mapped into the middle-lower part of the LG2 in 2017 and 2018 in both Guara and Nurlu maps. The highest LOD scores were 7.4 and 4.7, and the highest percentages phenotypic variations were 28.6% and 21.2% in 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 7.7 and 4.7, and the highest percentages of the explained phenotypic variations were 30.0% and 21.3% in 2017 and 2018, respectively. The closest marker in LG2 was pchgms1 in both years in both linkage maps (Table 4.17, Table 4.18 and Figure 4.60).

The other QTL in the 'Guara x Nurlu' population for KW was detected in the middle part of LG6 in the both maps in 2015 and 2018. The highest LOD scores were 4.1 and 4.0, and the highest percentages of the explained phenotypic variations were 18.5% and 18.7% in 2015 and 2018 in cv. Guara map, respectively. In the Nurlu map, the highest LOD scores were 4.0 and 4.1, and the highest explained phenotypic variations were 17.4% and 18.6% in 2015 and 2018, respectively. The closest marker in LG6 was pchgms5 in both years in both maps (Table 4.17, Table 4.18 and Figure 4.60).

Fernandez I Marti et al. (2013) detected QTL related to the kernel width on LG3 (4.6 LOD and 30.6% PV) and LG5 (2.4 LOD and 17.0% PV). In this study,

QTL related to kernel width mapped on LG2 (7.4 LOD and 28.6% PV) and LG6 (4.1 LOD and 18.5% PV) in 'Guara x Nurlu' population.

4.2.3.10. Kernel Thickness (KT)

In the 'Gülcan-2 x Lauranne' population, one significant QTL for Kernel Thickness (KT) was mapped into the middle-upper part of the LG4 in 2015, 2017 and 2018 in cv. Gülcan-2 map and in 2017 and 2018 in Lauranne map. The highest LOD scores were 3.3, 5.5 and 3.4, and the highest percentages of the explained phenotypic variations were 20.1%, 23.0% and 15.7% in 2015, 2017 and 2018 in the Gülcan-2 map, respectively. In cv. Lauranne map, the highest LOD scores were 5.5 and 3.4, and the highest explained phenotypic variations were 23.0% and 15.5% in 2017 and 2018, respectively. The closest marker in LG4 was UCD-CH15 in the both maps in all years (Table 4.16, Table 4.18 and Figure 4.59).

In the 'Guara x Nurlu' population, one significant QTL for Kernel Thickness (KT) was mapped into the middle-lower part of the LG8 in 2015, 2016, 2017 and 2018 in both maps. The highest LOD scores were 3.9, 5.2, 7.5 and 11.7, and the highest percentages of the explained phenotypic variations were 16.7%, 24.3%, 28.8% and 43.8% in 2015, 2016, 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 3.9, 5.2, 7.5 and 11.7, and the highest explained phenotypic variations were 16.7%, 24.3%, 29.0% and 43.8% in 2015, 2016, 2017 and 2018, respectively. The closest markers in LG8 were 7302701 (29.5 cM) in cv. Guara map and MA019a at 39.8 cM in the Nurlu map in all years (Table 4.17, Table 4.18 and Figure 4.60).

Fernandez i Marti et al. (2013) detected QTL related to the kernel thickness on LG6 (2.9 LOD and 24.2% PV) and LG7 (3.0 LOD and 16.1% PV). Font i Forcada et al. (2015) mapped kernel thickness in LG6 in almond association mapping study. In this study, QTL related to kernel thickness mapped on LG4 (5.5 LOD and 23.0% PV) in in 'Gülcan-2 x Lauranne' population, while mapped on LG8 (11.7 LOD and 43.8% PV) in 'Guara x Nurlu' population.

4.2.3.11. Kernel Weight (KWt)

In the 'Guara x Nurlu' population, one significant QTL for Kernel Weight (KWt) was mapped into the middle-lower part of the LG8 in all four years in the Guara maps and in 2017 and 2018 in cv. Nurlu maps. The highest LOD scores were 3.4, 2.9, 6.5 and 11.0, and the highest percentages of the explained phenotypic variations were 16.9%, 14.3%, 25.7% and 41.7% in 2015, 2016, 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 5.1 and 7.7, and the highest explained phenotypic variations were 22.4% and 33.0% in 2017 and 2018, respectively. The closest marker in LG8 was EPDCU3454 at 41.4 cM in the Guara map and MA019a (39.8 cM) in cv. Nurlu map in all years (Table 4.17, Table 4.18 and Figure 4.60).

Sanchez-Perez et al. (2007) mapped QTL related to kernel weight on LG1 (3.1 LOD and 13.4% PV) and LG2 (3.6 LOD and 14.0% PV). Fernandez I Marti et al. (2013) detected the QTL related to the kernel weight on LG7 (2.9 LOD and 14.4% PV). Font i Forcada et al. (2015) failed to map this trait on the linkage map. In this study, QTL related to kernel weight mapped on LG8 (11.0 LOD and 417% PV) in 'Guara x Nurlu' population.

4.2.3.12. Kernel Nut Ratio (KNR)

In the 'Guara x Nurlu' population, two significant QTLs were detected for Kernel Nut Ratio (KNR). One QTL was mapped into the lower part of the LG6 in 2016 and 2018 in both Guara and Nurlu maps. The highest LOD scores were 3.4 and 3.3, and the highest percentages of the explained phenotypic variations were 16.1% and 14.9% in 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 3.3 and 3.6, and the highest explained phenotypic variations were 15.7% and 16.5% in 2017 and 2018, respectively. The closest marker in LG6 was AMPA121 in both years in both loinkage maps (Table 4.17, Table 4.18 and Figure 4.60).

The other QTL in the 'Guara x Nurlu' population for KNR was identified in the middle-lower part of LG8 in both maps in all four years. The highest LOD scores were 3.9, 3.5, 7.2 and 7.4, and the highest percentages of the explained phenotypic variations were 18.8%, 17.0%, 28.5% and 30.7% in 2015, 2016, 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 3.9, 4.8, 6.9 and 5.8, and the highest explained phenotypic variations were 18.6%, 22.0%, 27.7% and 24.8% in 2015, 2016, 2017 and 2018, respectively. The closest marker in LG6 was EPDCU3454 (41.4 cM) in cv. Guara map and MA019a at 39.8 cM in the Nurlu map in all four years (Table 4.17, Table 4.18 and Figure 4.60).

4.2.3.13. Softness of Shell (SoS)

In the 'Gülcan-2 x Lauranne' population, one significant QTL for Softness of Shell (SoS) was mapped into the lower part of the LG5 in 2016 and 2018 in Gülcan-2 and Lauranne maps. The highest LOD scores were 3.9 and 4.4, and the highest percentages of the explained phenotypic variations were 20.8% and 16.9% in 2016 and 2018 in cv. Gülcan-2 map, respectively. In the Lauranne map, the highest LOD scores were 4.0 and 4.7, and the highest explained phenotypic variations were 22.4% and 21.0% in 2016 and 2018, respectively. The closest marker in LG5 was EPDCU4658 in all years in both maps (Table 4.16, Table 4.18 and Figure 4.59).

Softness of shell is a very important trait for almond. Soft-shelled cultivars are preferred by consumers while hard-shelled cultivars are better for protecting the kernel from the harm coming from outside. Arus et al. (1999) mapped QTL related to Softness of shell on LG8. Sanchez-Perez et al. (2007) mapped QTL related to Softness of shell on LG2 (5.6 LOD and 19.7% PV). Goonetilleke et al. (2018) mapped QTL related to Softness of shell on LG2 (3.4 LOD and 9.0% PV), LG5 (4.2 LOD and 11.0% PV) and LG8 (3.4 LOD and 9.0% PV). In this study, QTL related to shell hardness mapped on LG5 (4.4 LOD and 16.9% PV) in in 'Gülcan-2 x Lauranne' population.

4.2.3.14. Nut Shape (NS)

In the 'Guara x Nurlu' population, two significant QTLs were identified for Nut Shape (NS). One QTL was mapped into the middle part of the LG2 in 2017 and 2018 in both maps. The highest LOD scores were 3.2 and 4.0, and the highest percentages of the explained phenotypic variations were 13.3% and 17.7% in 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 3.3 and 4.4, and the highest explained phenotypic variations were 15.5% and 19.1% in 2017 and 2018, respectively The closest marker in LG2 was UDA-051 at 20.5 cM in Guara map and BPPCT004 (23.1 cM) in cv. Nurlu map in both years (Table 4.17, Table 4.18 and Figure 4.60).

The other QTL in the 'Guara x Nurlu' population for NS was detected in the middle part of LG7 in both maps in three growing seasons in 2015, 2017 and 2018. The highest LOD scores were 4.0, 5.7 and 3.0, and the highest percentages of the explained phenotypic variations were 17.7%, 25.8% and 15.1% in 2015, 2017 and 2018 in cv. Guara map, respectively. In the Nurlu map, the highest LOD scores were 4.3, 6.0 and 2.8, and the highest explained phenotypic variations were 18.2%, 24.3% and 13.2% in 2015, 2017 and 2018, respectively. The closest marker in LG7 was UDAp-407 in all three years in both maps (Table 4.17, Table 4.18 and Figure 4.60).

4.2.3.15. Kernel Color Intensity (KCI)

In the 'Guara x Nurlu' population, one significant QTL for Kernel Color Intensity (KCI) was mapped into the middle part of the LG8 in 2015 and 2018 in both Guara and Nurlu maps. The highest LOD scores were 5.9 and 4.0, and the highest percentages of the explained phenotypic variations were 25.1% and 17.6% in 2015 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 6.0 and 4.1, and the highest percentages of the explained phenotypic variations were 25.0% and 17.6% in 2015 and 2018, respectively. The closest marker in LG8 was PaCITA15 in all years in both maps (Table 4.17, Table 4.18 and Figure 4.60).

4.2.3.16. Shriveling of Kernel (SoK)

In the 'Gülcan-2 x Lauranne' population, one significant QTL for Shriveling of Kernel (SoK) was mapped into the upper middle part of the LG6 in 2015 and 2018 in both maps. The highest LOD scores were 5.5 and 3.3, and the highest percentages of the explained phenotypic variations were 34.5% and 13.3% in 2015 and 2018 in cv. Gülcan-2 map, respectively. In the Lauranne map, the highest LOD scores were 3.8 and 3.1, and the highest explained phenotypic variations were 20.1% and 15.1% in 2015 and 2018, respectively. The closest markers in LG6 were 7311880 at 10.6 cM in the Gülcan-2 map and AMPA100 (5.1 cM) in cv. Lauranne map in both years (Table 4.16, Table 4.17 and Figure 4.59).

In the 'Guara x Nurlu' population, one significant QTL for Shriveling of Kernel (SoK) was mapped into the lower part of the LG8 in 2015 and 2018 in both maps. The highest LOD scores were 4.1 and 4.6, and the highest percentages of the explained phenotypic variations were 17.4% and 20.3% in 2015 and 2018 in cv. Guara map, respectively. In the Nurlu map, the highest LOD scores were 3.0 and 3.7, and the highest explained phenotypic variations were 13.3% and 16.3% in 2015 and 2018, respectively. The closest markers in LG8 were 7298980 (43.1 cM) in cv. Guara map and 7310420 at 46.7 cM in the Nurlu map in all years (Table 4.17, Table 4.18 and Figure 4.60).

4.2.3.17. Double Kernel Ratio (DKR)

In the 'Gülcan-2 x Lauranne' population, two significant QTLs were detected for Double Kernel Ratio (DKR). One QTL was mapped into the middle-upper part of the LG1 in 2015 and 2016 in both maps. The highest LOD scores were 4.7 and 7.1, and the highest percentages of the explained phenotypic variations were 25.6% and 32.9% in 2015 and 2016 in the Gülcan-2 map, respectively. In cv. Lauranne map, the highest LOD scores were 5.8 and 5.6, and the highest explained phenotypic variations were 23.8% and 26.1% in 2015 and 2016, respectively The closest marker in LG1 was CPDCT038 (14.8 cM) in cv. Gülcan-2 map and

CPDCT027 at 24.6 cM in the Lauranne map in both years (Table 4.16, Table 4.18 and Figure 4.59).

The other QTL in the 'Gülcan-2 x Lauranne' population for DKR was detected in the middle part of LG6 in both maps in 2015 and 2018. In cv. Gülcan-2 map, the highest LOD scores were 3.7 and 3.8, and the highest explained phenotypic variations were 19.8% and 17.2% in 2015 and 2018, respectively. In the Lauranne map, the highest LOD scores were 3.7 and 3.8, and the highest explained phenotypic variations were 19.6% and 17.2% in 2015 and 2018, respectively. The closest marker in LG6 was CPSCT012 in both years in both linkage maps (Table 4.16, Table 4.18 and Figure 4.59).

In the 'Guara x Nurlu' population, three significant QTLs were detected for Double Kernel Ratio (DKR). One QTL was mapped into the middle part of LG1 in 2015 and 2018 in the Guara map and lower part of LG1 in the Nurlu map. The highest LOD scores were 3.8 and 3.1, and the highest percentages of the explained phenotypic variations were 16.5% and 14.3% in 2015 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 3.8 and 3.4, and the highest explained phenotypic variations were 16.4% and 15.8% in 2015 and 2018, respectively The closest marker in LG1 was 7315337 at 27.5 cM in the Guara map and 7301293 (47.5 cM) in cv. Nurlu map in both years (Table 4.17, Table 4.18 and Figure 4.60).

The second QTL in the 'Guara x Nurlu' population for DKR was detected on the lower part of LG6 in both maps in 2015 and 2018. In cv. Guara map, the highest LOD scores were 4.0 and 6.8, and the highest percentages of the explained phenotypic variations were 16.7% and 27.7% in 2015 and 2018, respectively. The highest LOD scores were 4.0 and 7.1, and the highest explained phenotypic variations were 16.7% and 28.9% in 2015 and 2018 in the Nurlu map, respectively. The closest marker in LG6 was AMPA121 in both years in both maps (Table 4.17, Table 4.18 and Figure 4.60).

The third QTL in the 'Guara x Nurlu' population for DKR was identified in the middle-upper part of LG8 in the Guara map in all years, and the middle part of LG8 in the Nurlu map in 2015 and 2018. The highest LOD scores were 4.5, 4.0, 3.3 and 6.6, and the highest percentages of the explained phenotypic variations were 19.2%, 19.4%, 18.0% and 26.7% in 2015, 2016, 2017 and 2018 in the Guara map, respectively. In cv. Nurlu map, the highest LOD scores were 4.4 and 6.6, and the highest explained phenotypic variations were 18.6% and 26.7% in 2015 and 2018, respectively. The closest marker in LG8 was UDAp-470 in both years in both linkage maps (Table 4.17, Table 4.18 and Figure 4.60).

Double kernel is not a favorable trait in almond, cultivars with low double kernel ratio were preferred by the producer. This trait can be affected by the low temperature during the blooming (Grasselly, 1972). Sanchez-Perez et al. (2007) mapped QTL related to double kernel on LG4 (9.1 LOD and 25.6% PV). In this study, QTL related to double kernel ratio mapped on LG1 (7.1 LOD and 32.9% PV) and LG6 (3.8 LOD and 19.8% PV) in 'Gülcan-2 x Lauranne' population, while mapped on LG1 (3.8 LOD and 16.5% PV), LG6 (6.8 LOD and 27.7% PV) and LG8 (6.6 LOD and 26.7% PV) in 'Guara x Nurlu' population.

A total of nine traits which responsible for 12 and 11 QTL regions were mapped in Gülcan-2 and Lauranne maps, respectively. Also, 23 QTL regions related to 15 traits were detected in Guara map and 14 traits responsible for 22 QTL regions were successfully mapped in cv. Nurlu map. Some of the QTL were detected for the first time in almond such as Shell L, Shell a, Kernel a, Kernel Nut Ratio, Nut Shape, Shell Color Intensity, Kernel Color Intensity and Shriveling of Kernel. We also detected some putative markers that closely related with the agronomically and commercially important traits with high explained phenotypic variations for almond such as: marker EPDCU3454 in LG8 for Kernel Nut Ratio and Kernel Weight, marker PaCITA15 in LG8 for Kernel Color Intensity and marker AMPA121 in LG6 and marker UDAp-470 in LG8 for Double Kernel Ration (Table 4.16 and Table 4.17).

In recent years, two almond genome sequences were released. The genome sequence of almond cv. Lauranne was released by Sanchez-Perez et al. (2019) and the genome sequence of almond cv. Texas was released by Alioto et al. (2020). With the help of these two almond genome sequences, we can conduct synteny analysis using our genetic linkage maps with these two almond genome sequences. We can also further investigate the genes or proteins that related or responsible to the agronomically important traits of interest. After further validation, some putative markers we detected in this study can be used in the future almond breeding programs in order to accelerate the breeding programs.

Table 4. 16. Summary of the QTLs in 'Gülcan-2 x Lauranne' F1 population.

	Year	Gülcan-2	GW	cM	LOD	PV%	Closest markers	Position	Kruskal-Walls test significance	Lauranne	GW	cM	LOD	PV%	Closest markers	Position	Kruskal-Walls test significance
Shell a	2017	LG4	2.8	4.7-5.9	3.2	14.5	pchgms2	5.9	******	LG4	2.7	5.1-7.5	3.8	17.4	pchgms2	7.5	******
	2018	LG4	2.9	4.7-6.9	3.2	14.7	pchgms2	5.9	****	LG4	2.9	6.1-7.5	3.2	14.5	pchgms2	7.5	****
Nut lenght	2016	LG2	3.1	37.5-41.8	3.5	17.5	UDP96-013	38.8	****	LG2	3	30.2-37.4	3.6	17.7	7303716	32.4	*****
	2017	LG2	2.9	37.5-38.5	3.1	13.7	UDP96-013	38.8	***	-	-	-	-	-	-	-	-
	2018	LG2	3	37.5-39.8	3.6	17.8	UDP96-013	38.8	****	LG2	3	31.2-34.4	3.3	15.4	7303716	32.4	*****
	2015	LG6	2.8	0-4.7	3.9	23.1	7301017	2.3	****	LG6	2.8	0-4	5.1	23.5	7312614	2	*****
	2017	LG6	2.8	0-4.3	4.3	19.8	7301017	2.3	*******	LG6	2.8	0-4	5.9	25.3	7312614	2	****
	2018	LG6	2.7	0-4.3	4.4	21.3	7301017	2.3	****	LG6	2.9	0-4	4.8	21.9	7312614	2	***
Nut Widht	2016	LG2	3.1	28.5-29.7	3.2	15.2	MA005a	29.7	****	LG2	3	20.5-22.4	3.2	15.2	MA005a	22.4	****
	2017	LG2	3	28.5-30.7	4.1	17.5	MA005a	29.7	****	LG2	3	20.5-23.4	4.1	17.5	MA005a	22.4	****
	2018	LG2	3.1	28.5-30.7	4.6	20.8	MA005a	29.7	******	LG2	2.8	20.5-23.4	4.6	21.0	MA005a	22.4	******
Nut Weight	2016	LG2	3	37.5-40.8	3.4	15.3	UDP96-013	38.8	**	-	-	-	-	-	-	-	-
	2017	LG2	3	37.5-41.8	4.1	16.8	UDP96-013	38.8	***	-	-	-	-	-	-	-	-
	2018	LG2	2.9	37.5-41.8	5.1	20.0	UDP96-013	38.8	****	-	-	-	-	-	-	-	-
	2015	LG5	2.9	40.7-47	3.4	17.0	EPDCU4658	41.1	****	LG5	2.9	50.9-55.7	3.4	16.9	7314035	54.7	******
	2018	LG5	2.9	40.7-43.1	3.7	17.6	EPDCU4658	41.1	****	LG5	3	50.9-55.7	5	20.0	7314035	54.7	*******
Kernel Lenght	2015	LG6	2.8	0-4.3	4.5	25.5	7301017	2.3	******	LG6	2.8	0-4	4.7	27.7	7312614	2	******
	2017	LG6	2.6	0-4.3	3.8	17.5	7301017	2.3	*******	LG6	2.8	0-4	6.4	29.4	7312614	2	*******
	2018	LG6	2.7	0-4.3	6.5	30.4	7301017	2.3	*******	LG6	3	0-4	8	31.5	7312614	2	*******
Kernel Thickness	2015	LG4	2.9	16.7-20.7	3.3	20.1	UCD-CH15	16.7	****	-	-	-	-	-	-	-	-
	2017	LG4	2.9	16.2-20.7	5.5	23.0	UCD-CH15	16.7	*******	LG4	2.8	18.9-21.8	5.5	23.0	UCD-CH15	19.8	*******
	2018	LG4	2.9	16.2-20.7	3.4	15.7	UCD-CH15	16.7	***	LG4	2.9	18.9-21.8	3.4	15.5	UCD-CH15	19.8	***
Softness of Shell	2016	LG5	2.7	40.7-43.1	3.9	20.8	EPDCU4658	41.1	****	LG5	3.1	50-53.9	4	22.4	EPDCU4658	50.9	****
	2018	LG5	2.9	40.7-43.1	4.4	16.9	EPDCU4658	41.1	*****	LG5	3	49-53.9	4.7	21.0	EPDCU4658	50.9	*****
SoK	2015	LG6	2.8	8.5-11.6	5.5	34.5	7311880	10.6	*****	LG6	2.9	4.1-6.1	3.8	20.1	AMPA100	5.1	****
	2018	LG6	2.8	8.5-10.6	3.3	13.3	7311880	10.6	***	LG6	2.6	4.1-6.1	3.1	15.1	AMPA100	5.1	*****
Double Kernel	2015	LG1	3.8	11.6-12.3	4.7	25.6	CPDCT038	14.8	**	LG1	3.8	24.6-28.9	5.8	23.8	CPDCT027	24.6	***
	2016	LG1	3.8	7.4-15.8	7.1	32.9	CPDCT038	14.8	******	LG1	4.1	23-28.6	5.6	26.1	CPDCT027	24.6	******
	2015	LG6	2.9	26.7-28.3	3.7	19.8	CPSCT012	28.3	******	LG6	2.9	25-28.1	3.7	19.6	CPSCT012	27.1	******
	2018	LG6	2.8	26.7-28.3	3.8	17.2	CPSCT012	28.3	***	LG6	2.9	25-28.1	3.8	17.2	CPSCT012	27.1	***

(**) p<0.01, (***) p<0.005, (****) p<0.001, (*****) p<0.0005,(******) p<0.0001, (*******)p<0.00005.

Table 4.17. Summary of the QTLs in 'Guara x Nurlu' F1 population.

Trait	Year	Guara	GW	cM	LOD	PV%	Closest markers	Position	Kruskal-Walls test significance	Nurlu	GW	cM	LOD	PV%	Closest markers	Position	Kruskal-Walls test significance
Shell L	2017	LG1	3	30.7-32.4	3.3	13.8	7305131	32.4	*******	-	-	-	-	-	-	-	-
	2018	LG1	2.9	29.7-32.4	3	16.4	7305131	32.4	*******	-	-	-	-	-	-	-	-
Kernel a	2015	LG8	2.7	14.4-15.4	3.2	13.8	7304874	14.4	****	LG8	3	27.7-32.2	3.8	16.3	UDAp-470	30.2	****
	2016	LG8	2.9	14.4-15.4	3	14.8	7304874	14.4	*****	LG8	2.7	25.6-27.5	3	14.5	MA042a	26.5	***
	2017	LG8	2.7	7.2-8.3	3.5	14.6	7307816	7.3	******	-	-	-	-	-	-	-	-
	2018	LG8	2.7	14.4-15.4	10.5	39.2	7304874	14.4	*******	LG8	2.9	27.7-32.2	10.5	39.9	UDAp-470	30.2	*******
Nut Lenght	2015	LG7	2.9	15.5-17.7	4.1	18.8	UDA-009	17.7	****	LG7	2.8	26.4-31.8	3.1	12.1	UDA-009	28.8	***
	2017	LG7	2.7	15.5-17.7	3.6	17.2	UDA-009	17.7	***	LG7	2.8	28.4-30.8	3.3	13.3	UDA-009	28.8	**
	2018	LG7	2.8	15.5-17.7	4.4	22.6	UDA-009	17.7	****	LG7	2.9	27.4-31.8	2.9	17.4	UDA-009	28.8	****
	2015	LG8	2.7	41.4-44.1	3.3	14.2	7298980	43.1	***	LG8	3.1	52.2-55.8	3.3	15.6	7300235	55.8	***
	2017	LG8	2.7	41.4-44.1	5.2	21.3	7298980	43.1	****	LG8	3.2	52.2-56.8	5.3	21.3	7300235	55.8	*******
	2018	LG8	2.9	41.4-44.1	4.7	20.6	7298980	43.1	****	LG8	3.4	52.2-56.8	4.8	20.8	7300235	55.8	******
Nut Width	2015	LG6	2.7	28.4-31.7	4.8	20.6	CPPCT023	29.7	*****	LG6	2.9	34.7-36	4.8	19.7	CPPCT023	35	******
	2016	LG6	2.8	29.4-30.7	3.2	15.9	CPPCT023	29.7	****	-	-	-	-	-	-	-	-
	2018	LG6	2.9	28.4-31.7	4.5	19.6	CPPCT023	29.7	******	LG6	2.9	34.7-36	4.5	19.1	CPPCT023	35	******
Nut Thickness	2015	LG6	2.9	28.4-31.7	4.3	19	CPPCT023	29.7	*****	LG6	2.9	34.7-36	4.9	20	CPPCT023	35	******
	2018	LG6	2.7	28.4-31.7	5.5	23	CPPCT023	29.7	*******	LG6	2.7	34.7-36	5.5	23	CPPCT023	35	*******
Kernel/Nut	2016	LG6	2.8	48.6-50.9	3.4	16.1	AMPA121	48.9	***	LG6	2.9	53.6-54.5	3.3	15.7	AMPA121	54.5	***
	2018	LG6	2.8	48.6-49.9	3.3	14.9	AMPA121	48.9	****	LG6	2.9	53.6-54.5	3.6	16.5	AMPA121	54.5	****
	2015	LG8	2.9	38.4-42.4	3.9	18.8	EPDCU3454	41.4	******	LG8	2.8	39.3-42.8	3.9	18.6	MA019a	39.8	***
	2016	LG8	2.8	38.4-42.4	3.5	17	EPDCU3454	41.4	****	LG8	2.8	38.3-42.8	4.8	22	MA019a	39.8	******
	2017	LG8	2.8	38.4-42.4	7.2	28.5	EPDCU3454	41.4	*******	LG8	3.8	38.3-42.8	6.9	27.7	MA019a	39.8	*******
	2018	LG8	2.9	38.4-42.4	7.4	30.7	EPDCU3454	41.4	*******	LG8	2.9	38.3-42.8	5.8	24.8	MA019a	39.8	*******
Nut Weight	2017	LG1	3	50.1-53	3.7	14.5	PMS67	52	****	LG1	3.2	66.3-70.5	4.5	17.4	PMS67	69.5	*****
	2018	LG1	3	49.1-53	4.3	14.2	PMS67	52	****	LG1	3	66.3-70.5	4.7	15.5	PMS67	69.5	*****
	2016	LG6	2.9	28.4-30.7	4.2	20.3	CPPCT023	29.7	******	LG6	2.9	34.7-36	3.8	18.1	CPPCT023	35	******
	2018	LG6	3	28.4-31.7	5.4	23.2	CPPCT023	29.7	*******	LG6	2.8	34.7-36	5.3	22.3	CPPCT023	35	*******
Kernel Lenght	2017	LG6	2.7	41.4-44.1	6.4	25.7	7298980	43.1	****	LG6	2.9	52.2-56.8	5.9	25.4	7300235	55.8	******
	2018	LG6	2.9	41.4-44.1	8.7	36.8	7298980	43.1	******	LG6	2.8	52.2-56.8	8.3	36.8	7300235	55.8	*******
	2015	LG8	2.8	14.4-16.7	5	21.4	MA042a	15.8	*******	LG8	3.1	25.6-27.5	5	20.9	MA042a	26.5	*******
	2016	LG8	2.7	14.4-16.7	3.7	18.2	MA042a	15.8	***	LG8	3	25.6-27.5	3.6	18	MA042a	26.5	***
	2017	LG8	2.8	14.4-16.7	4.1	16.8	MA042a	15.8	****	LG8	2.9	25.6-27.5	4.2	17.6	MA042a	26.5	****
	2018	LG8	2.8	14.4-16.7	4.1	18.2	MA042a	15.8	****	LG8	2.8	25.6-27.5	4.3	19.2	MA042a	26.5	****
Kernel Width	2017	LG2	2.9	35.2-40.8	7.4	28.6	pchgms1	36.8	*******	LG2	2.8	44.3-48.1	7.7	30	pchgms1	46.1	*******
	2018	LG2	2.8	35.2-40.8	4.7	21.2	pchgms1	36.8	*****	LG2	2.8	44.3-48.1	4.7	21.3	pchgms1	46.1	*****
	2015	LG6	2.8	26.8-29.4	4.1	18.5	pchgms5	28.4	******	LG6	3	35-38.1	4	17.4	pchgms5	36.1	*****
	2018	LG6	2.6	26.8-29.4	4	18.7	pchgms5	28.4	****	LG6	2.8	35-38.1	4.1	18.6	pchgms5	36.1	*****
	2015	LG8	2.8	27.7-30.5	3.9	16.7	7302701	29.5	******	LG8	2.8	38.3-42.8	3.9	16.7	MA019a	39.8	****

Kernel Thickness	2016	LG8	2.7	27.7-30.5	5.2	24.3	7302701	29.5	*******	LG8	3	38.3-42.8	5.2	24.3	MA019a	39.8	******
	2017	LG8	2.7	27.7-30.5	7.5	28.8	7302701	29.5	*******	LG8	2.8	38.3-42.8	7.5	29	MA019a	39.8	*******
	2018	LG8	2.7	27.7-30.5	11.7	43.8	7302701	29.5	*******	LG8	2.8	38.3-42.8	11.7	43.8	MA019a	39.8	*******
Kernel Weight	2015	LG8	2.7	40.3-42.4	3.4	16.9	EPDCU3454	41.4	****	-	-	-	-	-	-	-	-
	2016	LG8	2.8	40.3-41.4	2.9	14.3	EPDCU3454	41.4	******	-	-	-	-	-	-	-	-
	2017	LG8	2.7	38.3-42.4	6.5	25.7	EPDCU3454	41.4	*******	LG8	2.9	38.3-42.8	5.1	22.4	MA019a	39.8	******
	2018	LG8	2.7	38.3-42.4	11	41.7	EPDCU3454	41.4	*******	LG8	3.4	38.3-42.8	7.7	33	MA019a	39.8	*******
Nut Shape	2017	LG2	2.8	20.1-22.5	3.2	13.3	UDA-051	20.5	****	LG2	2.9	20-25.1	3.3	15.5	BPPCT004	23.1	****
	2018	LG2	2.9	18.1-22.5	4	17.7	UDA-051	20.5	****	LG2	2.9	20-25.1	4.4	19.1	BPPCT004	23.1	****
	2015	LG7	3	18.4-22	4	17.7	UDAp-407	19	***	LG7	2.8	28.8-33.8	4.3	18.2	UDAp-407	31.8	***
	2017	LG7	3	18.4-22	5.7	25.8	UDAp-407	19	*******	LG7	2.8	28.8-33.8	6	24.3	UDAp-407	31.8	*******
	2018	LG7	2.9	18.4-22	3	15.1	UDAp-407	19	**	LG7	2.8	28.8-30.8	2.8	13.2	UDAp-407	31.8	**
KCl	2015	LG8	2.7	20.1-22.1	5.9	25.1	PaCITA15	22.1	*******	LG8	3.2	30.2-34.8	6	25	PaCITA15	32.8	*******
	2018	LG8	2.9	20.1-22.1	4	17.6	PaCITA15	22.1	****	LG8	2.9	31.2-34.8	4.1	17.6	PaCITA15	32.8	****
SoK	2015	LG8	2.8	41.4-44.1	4.1	17.4	7298980	43.1	*******	LG8	2.8	45.2-48.7	3	13.3	7310420	46.7	**
	2018	LG8	3	41.4-44.1	4.6	20.3	7298980	43.1	******	LG8	2.8	43.2-48.7	3.7	16.3	7310420	46.7	*****
Double Kernel	2015	LG1	3.1	26.6-29.5	3.8	16.5	7315337	27.5	******	LG1	3.2	45.1-49.5	3.8	16.4	7301293	47.5	****
	2018	LG1	3	26.6-28.5	3.1	14.3	7315337	27.5	*******	LG1	3.1	45.1-48.5	3.4	15.8	7301293	47.5	***
	2015	LG6	2.8	48.6-52.7	4	16.7	AMPA121	48.9	******	LG6	3	53.6-54.5	4	16.7	AMPA121	54.5	******
	2018	LG6	3	48.6-52.7	6.8	27.7	AMPA121	48.9	*******	LG6	3	53.6-54.5	7.1	28.9	AMPA121	54.5	*******
	2015	LG8	2.8	18.2-19.8	4.5	19.2	UDAp-470	19.8	******	LG8	3.1	27.7-32.2	4.4	18.6	UDAp-470	30.2	******
	2016	LG8	2.7	18.2-19.8	4	19.4	UDAp-470	19.8	******	-	-	-	-	-	-	-	-
	2017	LG8	2.8	19.2-19.8	3.3	18	UDAp-470	19.8	****	-	-	-	-	-	-	-	-
	2018	LG8	2.8	18.2-19.8	6.6	26.7	UDAp-470	19.8	*******	LG8	2.9	27.7-32.2	6.6	26.7	UDAp-470	30.2	*******

(**) p<0.01, (***) p<0.005, (****) p<0.001, (*****) p<0.0005,(******) p<0.0001, (*******)p<0.00005.

Table 4.18. Distribution of different QTLs in the linkage groups of four parental linkage maps

Traits	Linkage Groups (LG)																															
	1				2				3				4				5				6				7				8			
	G2	L	G	N	G2	L	G	N	G2	L	G	N	G2	L	G	N	G2	L	G	N	G2	L	G	N	G2	L	G	N	G2	L	G	N
Shell L (SL)															X																	
Shell a (Sa)													X	X																		
kernel a (Ka)																															X	X
Nut Length (NLh)					X	X															X	X					X	X			X	X
Nut Width (NW)					X	X																	X	X							X	X
Nut Thickness (NT)																							X	X								
Nut Weight (NWt)			X		X	X															X	X	X	X								
Kernel Length (KLh)																					X	X	X	X							X	X
Kernel Width (KW)							X	X															X	X								
Kernel Thickness (KT)													X	X																	X	X
Kernel Weight (KWt)																															X	X
Kernel Nut Ratio (KNR)																							X	X							X	X
Softness of Shell (SoS)																	X	X														
Nut Shape (NS)					X	X																			X	X						
Kernel Color Intensity (KCI)																															X	X
Shriveling of Kernel (SoK)																					X	X									X	X
Double Kernel Ratio (DKR)	X	X	X	X																	X	X	X	X							X	X

G2: Gülcan-2, L: Lauranne, G: Guara, N: Nurlu

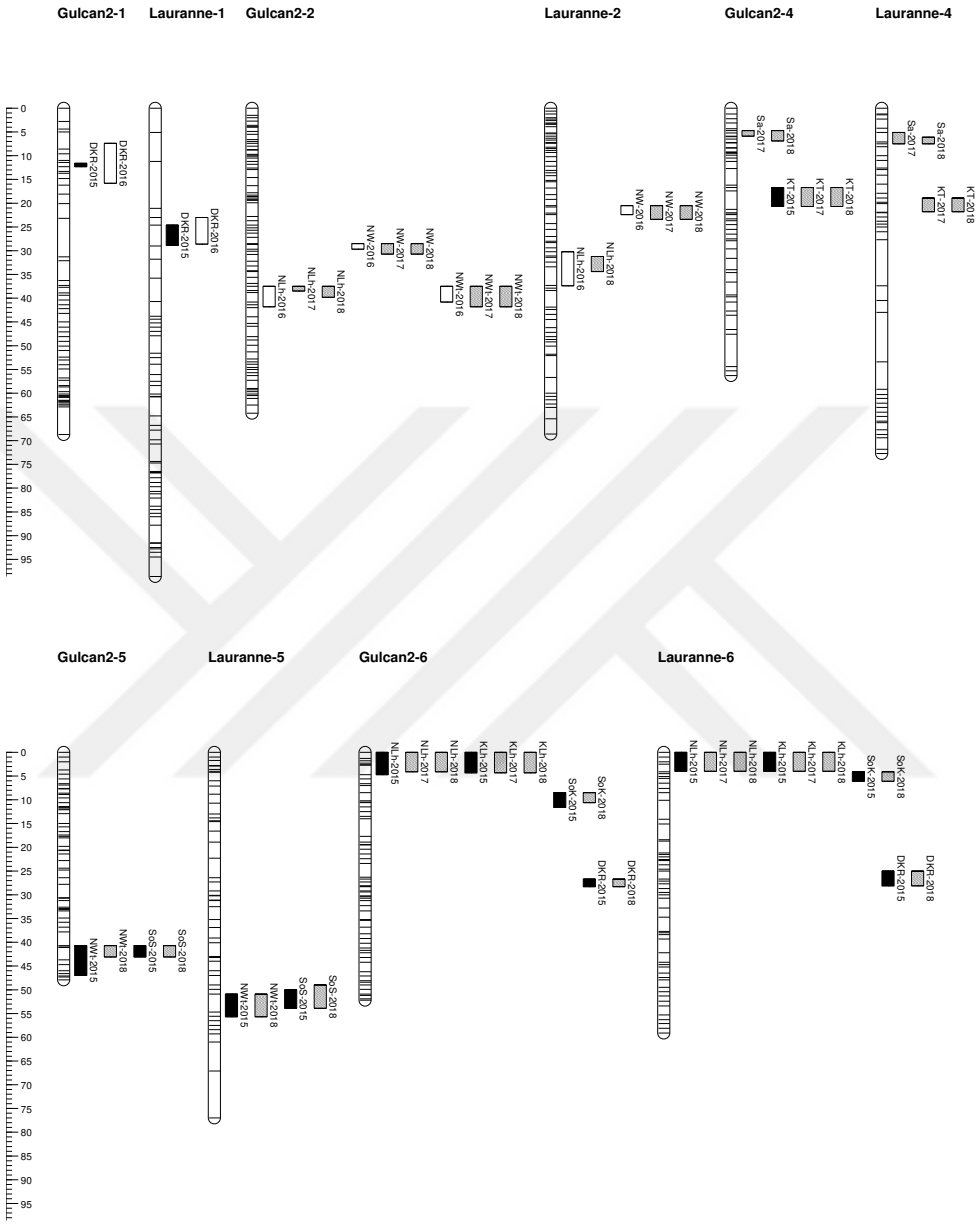

Figure 4.59. Gülcan-2 and Lauranne linkage maps with positions of QTLs related to the phenotypic traits. Having the significant QTLs in the five linkage groups (LG) 1, 2, 4, 5 and 6 of both parental maps. The black, white single striped and double striped bars on the right side of the LGs indicate the QTLs which are detected in 2015, 2016, 2017and 2018, respectively.

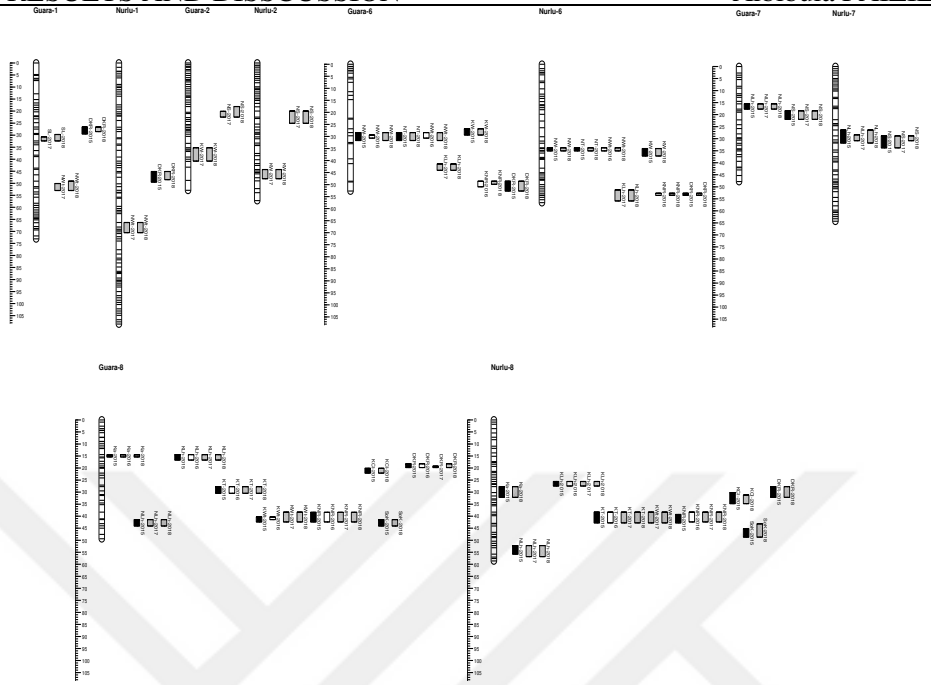

Figure 4.60. Guara and Nurlu linkage maps with positions of QTLs related to the phenotypic traits. Having the significant QTLs in the five linkage groups (LG) 1, 2, 6, 7 and 8 of both parental maps. The black, white single striped and double striped bars on the right side of the LGs indicate the QTLs which are detected in 2015, 2016, 2017and 2018, respectively.

5. CONCLUSIONS

In this study, we constructed four high-density parental linkage maps using 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' F1 populations by using DArT, SNP and SSR markers. A total of 107 and 114 individuals were used for map construction in 'Gülcan-2 x Lauranne' and 'Guara x Nurlu' populations, respectively. A total of 3,338 (2,125 DArT, 1,097 SNP and 116 SSR), 3,442 (2,128 DArT, 1,190 SNP, 124 SSR), 2,563 (1,210 DArT, 1,235 SNP and 118 SSR) and 3135 (1,334 DArT, 1669 SNP and 122 SSR) markers were successfully mapped in Gülcan-2, Lauranne, Guara and Nurlu maps, respectively. In both populations, phenotypic data related to nut and kernel characters such as Shell L (SL), Shell a (Sa), Shell b (Sb), Kernel L (KL), Kernel a (Ka), Kernel b (Kb), Nut Length (NLh), Nut Width (NW), Nut Thickness (NT), Nut Weight (NWt), Kernel Length (KLh), Kernel Width (KW), Kernel Thickness (KT), Kernel Weight (KWt), Kerbel Nut Ratio (KNR), Double Kernel Ratio (DKR), Softness of Shell (SoS), Nut Shape (NS), Shell Color Intensity (SCI), Kernel Color Intensity (KCI) and Shriveling of Kernel (SoK) were collected during four years (2015, 2016, 2017 and 2018). QTL analysis was conducted using the linkage maps and phenotypic data.

In Gülcan-2 map, the lengths of linkage groups ranged from 47.9 cM in LG5 to 68.7 cM in LG1. A total of 3,338 (2125 DArT, 1,097 SNP and 116 SSR) markers were mapped with total coverage of 469.9 cM in the maternal parent. An average of 417.3 markers was mapped in the female map with a mean marker density of 7.1. LG2 had the highest number of markers (611), while LG7 had the lowest (225). The highest average marker density was 9.5 in LG2, whereas the lowest density was 4.4 in LG7.

In Lauranne map, the lengths of linkage groups ranged from 59.1 cM in LG6 to 98.6 cM in LG1. A total of 3,442 (2,128 DArT, 1,190 SNP and 124 SSR) markers were mapped with total coverage of 572.3 cM in the paternal parent. An average of 430.3 markers was mapped in the female map with a mean marker density of 6.2.

LG2 had the highest number of markers (686), while LG5 had the lowest (329). The highest average marker density was 10.0 in LG2, whereas the lowest density was 3.7 in LG1.

In Guara map, the lengths of linkage groups ranged from 47.8 cM in LG7 to 73.1 cM in LG1. A total of 2,563 (1,210 DArT, 1,235 SNP and 118 SSR) markers were mapped with total coverage of 443.9 cM in the maternal parent. An average of 320.4 markers was mapped in the female map with a mean marker density of 5.8. LG2 had the highest number of markers (501), while LG6 had the lowest (70). The highest average marker density was 9.5 in LG2, whereas the lowest density was 1.3 in LG6.

In Nurlu map, the lengths of linkage groups ranged from 56.0 cM in LG3 to 108.5 cM in LG1. A total of 3135 (1334 DArT, 1669 SNP and 122 SSR) markers were mapped with total coverage of 516.0 cM in the paternal parent. An average of 391.9 markers was mapped in the female map with a mean marker density of 6.1. LG1 had the highest number of markers (610), while LG3 had the lowest (270). The highest average marker density was 8.7 in LG6, whereas the lowest density was 4.8 in LG3.

Among the 21 traits assessed in this study, most of the traits showed a normal distribution in all four years, while Double Kernel Ratio (DKR) presented skewed right distribution. As a result of QTL analysis, a total of 12, 11, 23 and 22 major QTLs which corresponded to 9, 9, 15 and 14 traits were detected in Gülcan-2, Lauranne, Guara and Nurlu maps, respectively. The QTLs for Kernel a, Kernel Weight and Kernel Color were located in LG8 in Guara and Nurlu maps. QTLs for Nut Length were located in LG2 and LG6 in Gülcan-2 and Lauranne maps, while they were mapped in LG7 and LG8 in Guara and Nurlu maps. The QTL of Nut Width was mapped in LG2 of Gülcan-2 and Lauranne maps, while it was located in LG6 of Guara and Nurlu maps. The QTL of Nut Thickness was located in LG6 of Guara and Nurlu maps. The QTLs for Nut Weight were located in LG2 and LG5 of Gülcan-2 and Lauranne maps, while it was mapped in LG2 of Guara map and in LG6 of both

Guara and Nurlu maps. The QTLs for Kernel Width were mapped in LG2 and LG6 of Guara and Nurlu maps. The QTLs of Kernel Thickness were located in LG4 of Gülcan-2 and Lauranne map, while it was mapped in LG8 of Guara and Nurlu maps. The QTLs for Kernel Nut Ratio was located in LG6 and LG8 of Guara and Nurlu maps. The QTLs of Softness of Shell were mapped in LG4 of Gülcan-2 and Lauranne maps. The QTLs of Nut Shape were located in LG2 and LG7 of Guara and Nurlu maps. The QTLs of Shriveling of Kernel were mapped in LG6 of Gülcan-2 and Lauranne mapsi while it was mapped in LG8 of Guara and Nurlu maps. The QTLs of Double Kernel Ratio were mapped in LG1 and LG6 of Gülcan-2 and Lauranne maps, while they were located in LG1, LG6 and LG8 of Guara and Nurlu maps.

These genetic linkage maps contructed in this study are the most high-density linkage maps in almond as well as in the *Prunus*. The studied nut and kernel chatacters are very important phenotypic traits in almond breeding programs. The high-density linkage maps constructed here are good resources for further genetic and genomic studies not only in almond but also in closely related species. Furthermore, the QTLs detected in this study may provide opportunities for marker assisted selection in almond cultivar breeding programs.

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