ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCE

PhD THESIS

Elmira ZIYA MOTALEBIPOUR

DEVELOPMENT OF SSR MARKERS BY NEXT GENERATION SEQUENCING IN *PISTACIA*, GENETIC LINKAGE MAPPING AND QTL ANALYSIS USING AN INTER-SPECIFIC F1 POPULATION

DEPARTMENT OF BIOTECHNOLOGY

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We certify that the thesis titled above was reviewed and approved for the award of degree of the PhD thesis by the board of Juries on 15/05/2017.

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ABSTRACT

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DEVELOPMENT OF SSR MARKERS BY NEXT GENERATION SEQUENCING IN *PISTACIA*, GENETIC LINKAGE MAPPING AND QTL ANALYSIS USING AN INTER-SPECIFIC F1 POPULATION

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Pistachio (*Pistacia vera* L.) is one of the most important nut crops in the world. There are about 11 wild species in the genus *Pistacia*. Genome survey sequences of pistachio was obtained at 40x depth by next generation sequencing to understand genome structure. The assembly of 26.77 Gb Illumina data produced 27,069 scaffolds in the *P. vera* cv. Siirt genome. A total of 59,280 SSR motifs were detected with a frequency of 8.67 kb. A total of 206 SSRs were used to characterize 24 pistachio cultivars and 20 wild *Pistacia* genotypes. The novel SSR loci developed from cultivated pistachio were highly transferable to wild *Pistacia* species. The SSR-based genetic linkage map was constructed using an inter-specific F1 population derived from *P. vera* L. 'Siirt' and monoecious *Pistacia atlantica* Desf. (Pa-18 genotype). A total of 388 SSR markers were mapped. The length of consensus map was 1,492 cM along with 15 linkage groups with an average marker distance of 3.7 cM. Sex-linked markers in the literature were also mapped with the SSR markers in LG1. Phenotypic data were collected during 2015 and 2016 growing season for five traits: leaf length (LL), leaf width (LW), number of leaflet (NLF), young shoot color (YSC), and leaf color (LC). QTL analysis were performed for the five traits using the the 'Siirt' and 'Pa-18' SSR-based genetic maps and 17 QTLs were identified. The novel SSR markers, linkage maps, and QTLs reported in this study will be useful resources for future genetic linkage maps and QTL analysis, and for identification of important genes in *Pistacia* as well as genome sequencing projects.

Keywords: Pistachio, *Pistacia*, SSR, genetic diversity, genetic map, QTL

ÖZ

DOKTORA TEZİ

YENI NESIL SEKANSLAMA TEKNOLOJISI KULLANILARAK Pistacia TÜRLERINDE SSR MARKÖRLERIN GELIŞTIRILMESI VE TÜRLERARASI F1 POPULASYONU KULLANILARAK GENETIK HARITALAMA VE QTL ANALİZLERİ

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Antepfıstığı (*Pistacia vera* L.), dünya sert kabuklu meyve üretimi içerisinde önemli bir yere sahiptir. *Pistacia* cinsi Anacardiaceae familyasının bir üyesi olup içerisinde on birden fazla tür bulunmaktadır. Antepfıstığının genom yapısını belirlemek için yeni nesil sekanslama teknolojisi kullanılarak *P. vera*'nın Siirt çeşidinde yaklaşık 40 kat yoğunluklu sekanslama yapılmış ve genomun bütünüyle ilgili bilgiler ortaya çıkarılmıştır. Sekanslama sonucunda toplam 26.77 Gb sekans verilerinin birleştirilmesi ile 27.069 adet dizi N_{50} = 3.4 kb olacak şekilde elde edilmiştir. Bunlar içerisinden 59.280 SSR motifi elde edilmiş ve 8.67 kb'de bir SSR tekrar dizisine rastlanmıştır. Toplam 206 SSR lokusu *Pistacia vera*'ya ait 24 adet antepfıstığı çeşidi ve 20 yabani *Pistacia* genotipini karakterize etmek amacıyla kullanılmıştır. Genetik haritalama çalışması Siirt x Pa-18 (monoik atlantik sakızı) F1 populasyonu kullanılarak yapılmıştır. Gerek bu çalışmada gerekse önce yapılan çalışmalarda geliştirilen SSR markörleri kullanılarak toplamda 388 SSR markörü 15 genetik bağlantı grubunda haritalanmıştır. Harita uzunluğu 1.492 cM ve her bir bağlantı grubuna düşen ortalama markör sayısı 3.7 cM olarak hesaplanmıştır. F1 bitkileri 2015 ve 2016 yıllarında yaprak uzunluğu, yaprak genişliği, yaprakçık çifti sayısı, yaprak rengi ve bir yıllık sürgün rengi bakımından karakterize edilmişlerdir. Yapılan QTL analizleri sonucunda yaprak ve sürgün özellikleri ile bağlantılı 17 adet QTL belirlenmiştir.

Anahtar Kelimeler: Antepfıstığı, *Pistacia*, SSR, genetik çeşitlilik, genetik haritalama, QTL

EXTENDED ABSTRACT

Pistachio (*Pistacia vera* L.) is one of the most important nut crops in the world. There are about 11 wild species in the genus *Pistacia*. The pistachio (*Pistacia vera* L.) was first cultivated in South Anatolia during the Hittites period, and due to high quality was introduced as a precious cultivar.

In the world, there are two gene centers of pistachio. The one is the Near East gene center which covers the high parts of Anatolia, Caucasia, Iran and Turkmenistan, and the other is the Central Asian gene center. The gene center of the culture forms of Pistachio are Anatolia, Iran, Syria and Afghanistan. Today, Pistachio is grown between 30-45 south-north parallels and is usually grown in the Northern Hemisphere.The most important producer countries of pistachio in the world are Iran, USA, Turkey, Syria and China respectively.

According to the data of FAO, the production amount of Iran was 478,600 tons in 2013 while the production quantities of USA, Turkey, China and Syria were 196,930, 88,600, 74,000 and 54,516 respectively. The most commercial common cultivars cultivated in Turkey are Siirt', 'Kirmizi', 'Uzun', 'Halebi' and 'Ohadi'. Cultivars such as 'Uzun', 'Kirmizi' and 'Halebi' are used in sweet and cake making and chocolate and confectionary industry, due to having long kernel with dark green color and also extraordinary taste and aroma. 'Siirt' and 'Ohadi' varieties are highly preferred as nut because they have a high rate of splitting and round shaped nuts.

Pistacia species are dioecious. Female and male flowers are on different trees. Pollination occurs with winds. Monoic *Pistacia* genotypes were also reported among *Pistacia* species. There are some problems in pollination and fertilization in dioecious fruit of pistachio. As in other nut shells fruit, edible part of pistachio is the seed, so it must be pollinated and fertilized. Flowers that are not fertilized are fall down or hollow fruits are formed.

Published information on the pistachio genome is limited. Therefore, a genome survey is necessary to obtain knowledge on the genome structure of pistachio by next generation sequencing. In this study, with next generation sequencing technology simple sequence repeat (SSR) markers were developed.

SSR markers have a high level of codominant and polymorphism. The identification of repeated base sequences in the genome and used them to developed primer pairs study is costly. SSR markers are codominant markers which can be recognized heterozygous and homozygous genotypes. Simple sequence repeat (SSR) markers are useful tools for germplasm characterization, genetic diversity analysis, and genetic linkage mapping, and may help elucidate genetic relationships among pistachio cultivars and species.

In molecular plant breding, selection of DNA marker method depends on the purpose of study, the structure of the population, the genome diversity studied, the polymorphism and reproducibility status, the time required for analysis, the investment and the cost of implementation. According to this, it is very important to evaluate the potential of the markers. However, genetic markers have ideal properties such as high polymorphism, being codominant, easily analyzing, reliability, containing lots of information, being economical, transferable and being suitable for automation.

The genetic linkage map is used to identified the location of genes on the chromosomes (locus). The plants genome are constructed with genetic linkage map. It is possible to map a chromosome or whole genome by mapping several genes and molecular markers and find their locations along the chromosome.

To explore the genome structure of pistachio, a genome survey was performed using the Illumina platform at approximately 40× coverage depth in the *P. vera* cv. Siirt. A total of 26.77 Gb clean data from 28.9 Gb raw sequences were used for K-mer analysis. The peak of the 17-mer distribution was about 28, and the total K-mer count was 16,684,162,450; therefore, the genome size of pistachio was estimated as 596 Mb. The assembly of 26.77 Gb Illumina data produced 27,069

scaffolds at N50 = 3.4 kb with a total of 513.5 Mb. A total of 59,280 SSR motifs were detected with a frequency of 8.67 kb. A total of 206 SSRs were used to characterize 24 pistachio cultivars and 20 wild *Pistacia* genotypes (four genotypes from each five wild *Pistacia* species) belonging to *P. atlantica, P. integerrima, P. chinenesis, P. terebinthus,* and *P. lentiscus* genotypes. Overall 135 SSR loci amplified in all 44 cultivars and genotypes, 41 were polymorphic in six *Pistacia* species. The novel SSR loci developed from cultivated pistachio were highly transferable to wild *Pistacia* species. A total of 2,036 alleles were produced by 206 SSR loci in the characterization of 44 *Pistacia* cultivars and genotypes, ranging from 2 to 19 per locus. The average He value ranged between 0.08 and 0.74. The PIC values ranged from 0.08 to 0.90 with an average of 0.71. The newly developed novel polymorphic SSRs in this study may help germplasm characterization, genetic diversity, and genetic linkage mapping studies in the genus *Pistacia*.

F2, F3, reverse hybrid, double haploid are used in selection of mapping populations. F1 populations are not widely used in perennial biopsies due to long life cycles. In the perennial plants, 'double pseudo testcross' method is used in genetic mapping studies in F1 populations. However, genetic map is separately constructed for each parent in this method. The number of haploid chromosomes of pistachio is n = 15. Therefore, the constructed genetic map must have 15 linkage groups.

In this study, a combined genetic linkage map was created using all the SSR markers developed to date in *Pistacia* species. The map counstructed using 388 SSR markers on 15 linkage groups in 126 F1 plants which were derived of crossing between *Pistacia vera* L. ‘Siirt’ and monoecious *Pistacia atlantica* Desf. (Pa-18 genotype). The total map length was 1,486.1 cM and the mean distance between the markers was 3.9 cM and the length of the linkage groups ranged from 41.6 cM (LG1) to 137.1 cM (LG2). The mean distance between the markers ranged from 1.5 cM (LG1) to 6.0 cM (LG14). The number of markers in the linkage

groups varied between 19 (LG8) and 32 (LG9). As a result, in this study SSR-based pistachio genetic map was created together with newly developed SSR markers.

In this study, two different genetic linkage maps were constructed by applying 'double pseudo-testcross' mapping method. From polymorphic segregation SSRs, 306 markers were mapped in 'Siirt' genetic map. The average number of markers per link group is calculated as 4.6. The genetic map of the 'Pa-18' genotype contained 285 SSR markers and the average number of markers per linkage group was 4.8. The 'Siirt' genetic map has a total length of 1,410.4 cM which spanned in 15 linkage groups and an average of marker density per linkage group were calculated as 25.7 cM whereas the 'Pa-18' genotype genetic map has a total of 1,362.5 cM lengths and an average of markers per linkage group 19.0 cM. As a result, the first genetic linkage map with SSR markers were performed with the 'Siirt' cultivar and monoecious *Pistacia atlantica* 'Pa-18' genotype.

In this study, the sex-related SSR markers which were developed from other studies in litreture were also mapped. The sex linked markers were mapped in LG1 between 0.0 cM and 26.5 cM. Twelve sex-linked SNP markers were located at the position of 26.3 cM, which was supposed to be the sex determining region. The consensus map of LG1 had 45 markers with a total length of 47.5 cM. The average distance between SSR markers was 1.05 cM and the average marker density was 0.94.

In this study, quantitative trait locus (QTL) studies were also conducted for the first time in pistachio. The genetic linkage map with SSR markers from crossing of *Pistacia vera* L. ‘Siirt’ and monoecious *Pistacia atlantica* Desf. (Pa-18 genotype) F1 population was used. Phenotype data were collected in 2014 and 2015 for five traits: leaf length (LL), leaf width (LW), number of leaflet (NLF), young shoot color (YSC), and leaf color (LC). QTL analyses were performed for the five traits using the the ‘Siirt’ and ‘Pa-18’ maps and 17 QTLs were identified. The QTLs of leaf length and leaf width were detected in LG2, 4 and 12 to ‘Siirt’ map and also were located on LG2, 4 to ‘Pa-18’ map. The QTL of number of

leaflets were detected in LG7 in both maps. The QTL of young shoot color were detected in LG9 in both map and the QTL of leaf color were detected in LG7 and 9 in both maps.

As a result, in this thesis, 1) the preliminary information of genome sequence has been obtained by revealing of pistachio genome structure; 2) new SSR markers were developed from *Pistacia vera* 3) Transferability of developed SSR markers to other Pistacia species was determined ,4) Phylogenetic analysis of *Pistacia* species were done using newly developed SSR markers; 5) Genetic maps were constructed with newly developed SSR markers and also existing SSR markers in literature using interspecific Siirt x monoecious *Pistacia atlantica* Desf. (Pa-18 genotype) F1 population in the, 6) Mapping of the sex-related markers developed in the literature, and 7) QTL analyzes were made for the first time in pistachio and quantitative trait loci related to leaf and shoot characteristics were determined.

GENİŞLETİLMİŞ ÖZET

Antepfıstığı (*Pistacia vera* L.), dünya fıstık üretimi içerisinde önemli bir yere sahiptir. *Pistacia* cinsi Anacardiaceae familyasının bir üyesi olup içerisinde on birden fazla tür bulunmaktadır. Antepfıstığının (*Pistacia vera* L.) ilk olarak Etiler döneminde Güney Anadolu'da kültüre alınıp yetiştirildiği ve kral sofralarına girecek kadar kaliteli kültür çeşitlerinin üretildiği bilinmektedir.

Dünyada antepfıstığının iki gen merkezi bulunmaktadır. Birisi; Anadolu, Kafkasya, İran ve Türkmenistan'ın yüksek kısımlarını içine alan Yakın Doğu gen merkezi, diğeri ise Orta Asya gen merkezidir. Antepfıstığının kültür formlarının gen merkezi ise Anadolu, İran, Suriye ve Afganistan olarak bilinmektedir. Antepfıstığı günümüzde 30-45 güney-kuzey paralelleri arasında ve genellikle kuzey yarım kürede yetiştirilmektedir

Dünya'da en önemli üretici ülkeler sırasıyla İran, ABD, Türkiye, Suriye ve Çin'dir. FAO'nun verilerine göre İran'ın üretim miktarı 2013 yılında 478.600 ton iken ABD, Türkiye, Çin ve Suriye'nin üretim miktarları sırasıyla 196.930, 88.600, 74.000 ve 54.516 tondur. Türkiye'de yaygın olarak 'Siirt', 'Kırmızı', 'Uzun', 'Halebi' ve 'Ohadi' çeşitlerinin yetiştiriciliği yapılmaktadır. Bunlardan 'Uzun', 'Kırmızı' ve 'Halebi' çeşitleri uzun daneli, iç renginin koyu yeşil olması ve olağanüstü tat ve aromasından dolayı özellikle tatlı ve pasta yapımı ile çikolata ve şekerleme sanayisinde kullanılmaktadır. 'Siirt' ve 'Ohadi' çeşitlerinin ise çıtlaklık oranının yüksek, yuvarlak daneli ve iri olması sebebiyle özellikle çerezlik olarak tercih edilmektedir.

Pistacia cinsine giren türler dioik olup dişi ve erkek çiçekler farklı ağaçlar üzerinde bulunmaktadır. Tozlanma rüzgarlarla gerçekleşir. *Pistacia* türlerinde monoik *Pistacia* genotiplerine de rastlanmıştır. Antepfıstığının dioik bir meyve olması tozlanma ve döllenmede bazı problemlere neden olabilmektedir. Diğer sert kabuklu meyvelerde olduğu gibi antepfıstığında da meyvenin yenilen kısmı tohumu

olduğundan, meyve eldesi için tozlanma ve döllenme zorunludur. Döllenmeyen çiçekler dökülür veya bunlardan içi boş (fıs) meyveler meydana gelir.

Antepfıstığının genomuyla ilgili yayınlanmış bilgiler sınırlıdır. Bu nedenle, yeni nesil sekanslama teknolojisi kullanılarak kısa sürede antepfıstığı genom yapısı hakkında bilgi elde edilmesi gerekmektedir. Bu çalışmada yeni nesil sekanslama teknolojisi ile SSR geliştirme yöntemi ile basit dizi tekrarlama (SSR) markörleri geliştirilmiştir.

SSR markörin kodominant ve polimorfizm seviyesi yüksektir. Analiz edilen genomdaki tekrarlanan baz dizilerinin belirlenmesi, bunların primer olarak kullanılması ön bir çalışmayı gerektirdiğinden dolayı maliyeti oldukça yüksektir. SSR markörleri, heterozigot ve homozigot genotipleri birbirinden ayırabilecek kodominant markörlerdir. SSR markörleri germplazm karakterizasyonu, genetik çeşitlilik analizi ve genetik bağlantı haritası için yararlı araçlar olup, antepfıstığı çeşitleri ve türleri arasındaki genetik ilişkilerin aydınlatılmasına yardımcı olabilir.

Moleküler bitki ıslahında DNA markör yönteminin seçimi; araştırmanın amacına, populasyonun yapısına, çalışılan türün genomik çeşitliliğine, polimorfizm ve tekrarlanabilirlik durumuna, analiz için gerekli zamana, yatırım ve uygulama maliyetine göre değişir. Buna göre markörlerin kullanım potansiyelini değerlendirmek çok önemlidir. Bununla birlikte genetik markörler, yüksek polimorfizm göstermesi, kodominant olması, kolayca analiz edilmesi, güvenirlilik, birçok bilgi içermesi, ekonomik olması, transfer edilebilme ve otomasyona uygun olma gibi ideal özelliklere sahip olmalıdır.

Genetik bağlantı haritası, genlerin kromozomlar üzerindeki yerlerinin (lokus) tespit edilmesinde kullanılır. Bu gen haritası ile bitkilerin genomu ortaya çıkarılır. Harita oluşturulurken moleküler markörler kullanılmaktadır. Birçok genin ve moleküler markörlerin birbirlerine göre kromozom boyunca diziliş sırasının haritalanması ile bir kromozomun veya tüm genomun haritasını çıkarmak mümkündür.

Antepfıstığının genom yapısını belirlemek için yeni nesil sekanslama teknolojisi kullanılarak *P. vera*'nın Siirt çeşidinde yaklaşık 40 kat yoğunlukta sekanslama yapıldı. Elde edilen diziler kullanılarak K-mer analizi yapıldı ve 17-mer dağılımının uç noktası yaklaşık 28 ve toplam K-mer sayısı 16.684.162.450 olarak belirlenmiştir. Bunun sonucu olarak ise antepfıstığının genom büyüklüğü 596 Mb olarak hesaplanmıştır. Sekanslama sonucunda toplam 26.77 Gb uzunluğundaki sekans verilerinden 27.069 sekans dizileri N50 = 3.4 kb olacak şekilde üretilmiştir. Bunlar içerisinden 59.280 SSR motifi elde edilmiş ve 8.67 kb'de bir SSR tekrar dizisine rastlanmıştır. Toplam 950 adet dizayn edilen SSR markörlerinden 206 SSR lokusu *Pistacia vera'y*a ait 24 adet antepfıstığı çeşidi ve 20 yabani (*Pistacia P. atlantica, P. integerrima, P. chinenesis, P. terebinthus* ve *P. lentiscus*) genotipini (her bir yabani *Pistacia* türünden dört genotip) karakterize etmek amacıyla kullanılmıştır. Toplam 135 SSR markörünün 44 *Pistacia* türünde amplifiye olduğu saptanmıştır. Bunlardan 41 adet markörün tüm *Pistacia* türünlerinde polimorfik olduğu belirlenmiştir. Ayrıca her bir lokus için allel sayısı 2-19 adet arasında değişmiş ve 206 SSR lokusundan toplamda 2,036 adet allel elde edilmiştir. Her bir lokus için beklenen heterozigotluğun 0.08 ile 0.74 değerleri arasında değişiklik gösterdiği belirlemiştir. Polimorfizm bilgi içeriği (PBİ) değeri ise 0.08 ile 0.90 arasında değişmiş ve ortalama 0.71 olarak belirlenmiştir. Bu çalışmayla yeni geliştirilmiş polimorfik SSR markörlerinin, *Pistacia* cinsinde germplazm karakterizasyonu, genetik çeşitlilik ve genetik haritalama çalışmalarında kullanılabileceği ortaya konulmuştur.

Haritalama populasyonlarının seçiminde çoğunlukla F2, F3, geri melez, double haploid gibi popülasyonlar kullanılmakta olup, F1 populasyonları ise çok yıllık bitkilerde normal yaşam döngülerinin çok uzun zaman almasından dolayı yaygın olarak kullanılmaktadır. Çok yıllık bitkilerde F1 populasyonları ile yapılan genetik haritalama çalışmalarında 'double pseudo testcross' yöntemi kullanılmakta olup bu yöntemde her bir ebeveyn için ayrı ayrı genetik harita oluşturulmaktadır.

Antepfıstığının haploid kromozom sayısı n=15'dir. Bu nedenle, oluşturulacak genetik haritanın 15 bağlantı grubuna sahip olması beklenir.

Bu çalşmada, *Pistacia* türünde bugüne kadar geliştirilen tüm SSR markörleri kullanılarak birleştirilmiş genetik bağlantı haritası oluşturulmuştur. 'Siirt' çeşidi ve monoik *Pistacia atlantica* 'Pa-18' genotipi arasındaki melezlemeye ait 126 F1 bitkisi kullanılarak 388 SSR markörü 15 bağlantı grubu üzerinde haritalanmştır. Toplam harita uzunluğu 1,486.1 cM ve markörler arasındaki ortalama uzaklık 3.9 cM olarak belirlenmiş olup bağlantı gruplarının uzunluğu 41.6 cM (LG1) ile 137.1 cM (LG2) arasında değişmiştir. Markörler arası ortalama mesafe 1.5 cM (LG1) ile 6.0 cM (LG14) arasında değişmiştir. Bağlantı gruplarındaki markör sayısı 19 (LG8) ile 32 adet (LG9) arasında değişiklik göstermiştir. Sonuç olarak, bu çalşmada yeni geliştirilen SSR markörleri ile birlikte SSR tabanlı antepfıstığı genetik haritası oluşturulmuştur.

Bu çalışmada ayrıca 'double pseudo-testcross' haritalama metodu uygulanarak iki ayrı genetik bağlantı haritası oluşturulmuştur. Açılım gösteren SSR'lardan 306 markör 'Siirt' çeşidi genetik haritasında haritalanmıştır. Her bir bağlantı grubuna düşen ortalama markör sayısı 4.6 olarak hesaplanmıştır. 'Pa-18' genotipine ait genetik harita toplamda 285 SSR markörü içermiş ve her bir bağlantı grubuna düşen ortalama markör sayısı 4.8 adet olmuştur. 'Siirt' çeşidi genetik haritası toplamda 15 bağlantı grubunda 1,410.4 cM uzunluğa ve bağlantı grubu başına ortalama 25.7 cM markör yoğunluğuna sahip olurken, 'Pa-18' genotipi genetik haritası ise toplamda 1,362.5 cM uzunluğa ve bağlantı grubu başına ortalama 19.0 cM markör haritalanmıştır. Sonuç olarak, bu çalışmayla antepfıstığının 'Siirt' çeşiti ve monoik *Pistacia atlantica* 'Pa-18' genotipin SSR markörleri ile yapılan ilk genetik bağlantı haritası oluşturulmuştur.

Bu çalışmada SSR markörleri birlikte daha önce başka çalışmalarda geliştirilen cinsiyet ile ilişkli markörler de haritalanmıştır. Cinsiyet ile ilişkili 16 adet SNP markörü LG1'de 0.0 cM ile 26.5 cM arasında haritalanmıştır. Bu markörlerden 12 adedi bağlantı grubunun 26.3 cM'da konumlandığı için bu bölge

cinsiyet belirleme bölgesi olarak hipotez edilmiştir. Tüm markörler ile oluşturulan LG1'de toplam 45 markör haritalanmış ve uzunluğu 47.5 cM olmuştur. Markörler arası mesafe 1.05 cM ve ortalama markör yoğunluğu ise 0.94 olarak hesaplanmıştır.

Bu araştırmada ayrıca antepfıstığında ilk defa Siirt x monoik Atlantik sakızı (Pa-18) F1 populasyonu ve SSR markörleri ile oluşturulan genetik harita kullanılarak kantitatif özellik lokus (QTL) çalışması da yapılmıştır. F1 bitkileri 2015 ve 2016 yıllarında yaprak uzunluğu, yaprak genişliği, yaprakçık çifti sayısı, yaprak rengi ve bir yıllık sürgün rengi bakımından karakterize edilmişlerdir. Yapılan QTL analizleri sonucunda yaprak ve sürgün özellikleri ile bağlantılı 17 adet QTL belirlenmiştir. Yaprak uzunluğu özelliği ile ilgili QTL, Siirt ve 'Pa-18' ait her iki haritada da hem LG2 hem de LG4'de belirlenmiştir. Yaprak genişliği özelliği ile ilgili QTL, 'Siirt' haritasında LG2 ve LG4 bağlantı gruplarında yer alır iken 'Pa-18' haritasında ise LG2, LG4 ve LG12 bağlantı gruplarında saptanmıştır. Yaprakçık sayısı ve yaprak rengi ile ilgili olan QTL'ler ise 'Siirt' ve 'Pa-18' haritalarında LG7'de yer almıştır. Sürgün rengi ile ilgili QTL ebeveyn haritalarında LG9'da belirlenmiştir.

Sonuç olarak, bu tez kapsamında 1)antepfıstığının genomunun yapısı ortaya çıkarılarak tüm genom dizisinin eldesi için ön bilgilere ulaşılmış, 2)P. vera'dan yeni SSR markörler geliştirilmiş, 3)Geliştirilen SSR markörlerin diğer Pistacia türlerine aktarılabilirliği belirlenmiş, 4)Yeni geliştirilen SSR markörler kullanılarak *Pistacia* türlerinin filogenetik analizi yapılmış, 5)Literatürde var olan ve yeni geliştirilen SSR markörler ve türlerarası Siirt x monoik *P. atlantica* (Pa-18) F1 populasyonu kullanılarak SSR markörlerle genetik haritalar elde edikmiş, 6)Literatürde geliştirilen cinsiyet ile ilişkili markörlerin haritalaması yapılmış ve 7)antepfıstığında ilk defa QTL analizleri yapılarak yaprak ve sürgün özellikleri ile ilişkili kantitatif özellik lokusları belirlenmiştir.

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LIST OF ABBREVIATONS

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
SRAP	: Sequence-Related Amplified Polymorphism
SAMPL	: Selectively Amplified Microsatellite Polymorphic loci
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
LL	: Leaf Length
LW	: Leaf Width
NLL	: Number of Leaflets
YSC	: Young Shoot Color
LC	: Leaf Color

1. INTRODUCTION

Pistacia is a genus of flowering plants in the cashew family, Anacardiaceae, which also contains mango, poison ivy, poison oak, pepper tree, and sumac plants (Kafkas, 2006a). The *Pistacia* genus consists of at least 11 species and is estimated to be about 80 million years old (Parfitt and Badenes, 1997). The pistachio is native to the arid zones of Central Asia; it has been cultivated for 3,000–4,000 years in Iran and was introduced into Mediterranean Europe by the Romans at the beginning of the Christian era (Crane, 1978). Pistachio cultivation extended westward from its center of origin to Italy, Spain, and other Mediterranean regions of Southern Europe, North Africa, and the Middle East, as well as to China and more recently to the United States and Australia (Hormaza et al, 1994, 1998).

Currently, Iran, The United States of America, Turkey, China and Syria are the main pistachio producers in the world. As reported by the latest statistics of the World Food and Agriculture Organization (FAO), the pistachio production in the world reached to 990.921 metric tons (MTs) in 2015-2016 and the highest production provided by Iran (478.600 MTs) and followed by USA (196.930 MTs), Turkey (88.600 MTs), China (74.000 MTs) and Syria (54.516 MTs; Faostat, 2016). According to the Administrative Committee (ACP) in USA, the pistachio production was 199,447.46 MTs in 2016 (http://acpistachios.org). According to Iran Pistachio Association (IPA) in 2016, pistachio production was obtained 170,000 MTs (http://iranpistachio.org), and it was 170.000 MTs in Turkish Istatistic Institute (http://tuik.gov.tr).

The main pistachio cultivation area Turkey is in the Southeast Anatolia region, especially Gaziantep, Şanlıurfa, Siirt, and Kahramanmaraş provinces, which cover 90–95% of pistachio production. Moreover, wild *Pistacia* species such as *Pistacia atlantica* Desf., *P. eurycarpa*, Yalt., *P. lentiscus* L., and *P. terebinthus* L., expanded in almost all parts of Anatolia. Other well-known *Pistacia* species in the

world are *P. integerrima* Stewart and *P. chinensis* Bunge. (Kafkas, 2006b). Pistachios are long-living plants with a juvenile period of approximately 5–10 years. In addition, wild *Pistacia* species have edible seeds and are used as rootstock seed sources for cultivated *P. vera* and they have been used for fruit consumption, oil extraction, soap production and as forest trees as well (Kafkas et al, 2002a).

All species in *Pistacia* are dioecious, however monoecious individuals within *P. atlantica* were also reported (Kafkas et al, 2000). Dioecy character and a long juvenile period are the primary difficulties encountered in breeding and genetic studies of *Pistacia*. The long juvenile period in combination with dioecious character causes large investments of time and land for characterization and evaluation of progenies in a breeding program. Furthermore, the genetic control of the most economically significant traits is not clearly understood, including disease and pest resistance, nut quality and sex determination mechanism in *Pistacia* (Türkeli and Kafkas, 2013). Therefore, molecular markers are good facilities to overcome such difficulties in the breeding programs.

Allelic forms of genes or genetic loci determine the biological structures which are known as genetic markers in plant breeding. These genes can be transmitted within generations, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene. There are two categories of markers which are used in genetics and plant breeding: classical markers and DNA markers. Classical markers include morphological, cytological and biochemical markers. Southern blotting, polymerase chain reaction (PCR), and DNA sequencing are based on different polymorphism-detecting DNA markers (Collard et al, 2005; Jiang, 2013).

DNA markers or molecular markers play a major role in breeding programs. The criteria for defining ideal molecular markers based on application and species involved are: 1) high level of polymorphism, 2) even distribution across the whole genome (not clustered in certain regions), 3) co-dominance in expression which make heterozygosity distinguishable, 4) clear distinct allelic

features (so that the different alleles can be easily identified), 5) single copy and no pleiotropic effect, 6) low cost to use (or cost-efficient marker development and genotyping), 7) easy assay/detection and automation, 8) high availability and suitability which can be accumulated and shared among laboratories, 10) no harmful effect on phenotype (Jiang, 2013).

Several molecular markers such as randomly amplified polymorphic DNA (RAPD; Hormaza et al, 1994, Kafkas and Perl-Treves, 2001a;b), simple sequence repeats (SSR; Zaloglu et al, 2015), sequence-related amplified polymorphism (SRAP; Ahmad et al, 2005), amplified fragment length polymorphism (AFLP); (Kafkas, 2006a), inter-simple sequence repeats (ISSR; Kafkas et al, 2006b), and selectively amplified microsatellite polymorphic loci (SAMPL; Karimi and Kafkas, 2011), and single nucleotide polymorphisms (SNP; Kafkas et al, 2015) have been used to assess the genetic diversity, fingerprinting, phylogenetic relationships, germplasm characterization, sex determination, and genetic linkage mapping in cultivated and wild *Pistacia* species.

SSRs are useful as molecular markers and very polymorphic due to the high mutation rate affecting the number of repeat units (Levinson and Gutman, 1987). They are very useful for assaying diversity in natural populations or germplasm collections, and for fingerprinting and parental identification. They are also very valuable markers especially for genetic linkage mapping and evolutionary studies (Varshney et al, 2005) and have a high level of transferability between closely related species. The development of SSR markers from *P. vera* (Ahmad et al, 2003; Kolahi-Zonoozi et al, 2014; Zaloglu et al, 2015; Topcu et al, 2016; Khodaeiaminjan et al, 2017a) and from wild *Pistacia* species were performed in several studies (Albaladejo et al, 2008; Arabnezhad et al, 2011, Chen et al, 2011), however there are a limited number of SSR markers in the literature and they are not adequate for complet genetic linkage mapping in pistachio.

Pistachio is a diploid plant which has a haploid chromosome number of n=15 and also has high degree of heterozygosity, so it is considered as a proper

model for genetic linkage mapping studies in which inter- and intraspecific hybrids can be easily produced (Kafkas, 2003). F1 progenies derived from crosses between two heterozygous parents are typically used for genetic mapping studies in outbreeding heterozygous tree species. So by analyzing the markers and the double pseudo-testcross strategy, linkage maps can be constructed (Grattapaglia and Sederoff, 1994).

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 maps. Next generation sequencing (NGS) has provided a new perspective for research, owing to its high throughout and speed of data generation. It is also allows identifying the large numbers of microsatellites which causes to find variable number of tandem repeat (or VNTR) locations in a genome where a short nucleotide sequence is organized as a tandem repeat. They are easy to use, highly reproducible, multiallelic, codominant, relatively abundant, and widely distributed in most plant genomes. Each variant acts as an inherited allele, allowing them to be used for parental identification (Zalapa et al, 2012). This technology mainly suitable model for genetic and QTL mapping, and also significant key to study complex traits (Capobianchi et al, 2013). So far, NGS has been applied to genomics-based strategies to discover sequences for novel SSR markers in plants, with low cost (Capobianchiet et al, 2013). SSR development studies from a genome survey were performed in different plant species (Sonah et al, 2011; Jiao et al, 2012; Wei et al, 2014). Genome survey studies provide information about genome structure of a plant species, including estimates of genome size, levels of heterozygosity, and repeat contents. A study by Horjales et al (2003) is the only one in the literature to estimate genome size in the genus *Pistacia.* The genome size of *P. terebinthus* was estimated to be 2C = 1.32 Gb by flow cytometry.

NGS technology is a good option to develop novel SSR markers in plant in a short time with a low cost. *De novo* next generation sequencing reveals abundant number of SSR markers in a species without whole genome sequencing. SSR development studies using NGS technology were performed in different plant species (Csencsics et al, 2010; Iorizzo et al, 2011; Zhu et al, 2012; Gilmore et al, 2013; Park et al, 2013; Celik et al, 2014; Xiao et al, 2015; Hou et al, 2016). Khodaeiaminjan et al (2017b) used four *Pistacia* genotypes including; 'Siirt' 'Ohadi', 'Bağyolu' and 'Pa-18' to develop polymorphic SSR markers in pistachio using NGS technology. The authors designed 750 polymorphic SSR primer pairs *in silico* between four genotypes.

Recently, genetic structure analyses have focused on the collection, protection, and utilization of germplasm for a plant species (Emanuelli et al, 2013; de Jesus et al, 2013). It is important to explore population structure to avoid false genetic trends and to identify cultivars with specific or minor alleles that was important for molecular breeding programs (Liu et al, 2015). However, information about population structure of *Pistacia* collections assessed using a large and comprehensive set of SSR markers is limited in the litreture.

The first genetic linkage map was constructed in pistachio by Türkeli and Kafkas (2013) who used an inter-specific F1 population having 92 individuals derived from a cross between *P. vera* cv.'Siirt' and *P. atlantica* (monoecious genotype 'Pa-18'). The authors used AFLP, SRAP, and ISSR markers, and 17 linkage groups in 'Siirt' and 19 linkage groups in 'Pa-18' genotype were constructed. Insufficient number of markers or individuals is most probably to reduced recombination frequency and created minor linkage groups which cannot link to a major linkage. The authors suggested using more markers with more individuals to resolve the data into 15 linkage groups. The second mapping study was performed by Khodaeiaminjan et al (2017b) who mapped 385 SSRs using Siirt x Bağyolu intra-specific F1 population with 122 progenies.

This study aim to (1) estimate the genome size, GC content, and heterozygosity rates of pistachio (*P. vera* cv. Siirt) using a genome survey, (2) to perform genome-wide characterization of SSRs in the *P. vera* genome, (3) to develop novel SSR markers for pistachio, (4) to determine transferability of SSR markers to other *Pistacia* species, (5) to reveal the population structure of *Pistacia* germplasm, (6) to construct genetic linkage maps using an inter-specific F1 population, (7) to determine positions of previously identified sex-linked markers, and (8) to perform QTL analysis of leaf and shoot traits in pistachio.

2. LITERATURE REVIEW

2.1. Characterization of Pistachio *(P. vera* L.*)* Cultivars By Molecular Markers

The first cultivar characterization study by DNA markers in pistachio was published by Hormaza et al (1994) who used RAPD markers to examine the relationship between 15 pistachio cultivars. The authors separated 15 cultivars into two major clusters: Mediterranean cluster which included cultivars from Mediterranean region, Europe, North Africa and Middle East, and Iranian-Caspian cluster that contained cultivars from the east of Zagros Mountains.

Hormaza et al (1998) used fifteen pistachio cultivars with 14 RAPD primers and clustered them according to their geographical origin. Two major clusters were determined: one comprising cultivars originated in the Mediterranean countries and the other from Iran and the Caspian Sea.

Caruso et al (1998) conducted morphological description and RAPD fingerprinting analysis on 49 male and 44 female accessions originating in Mediterranean and Central Asian countries, and collected from Italy, Greece, Morocco, Spain and Turkey. On the whole, a high degree of polymorphism was detected on both the phenotypic and genetic traits examined. Among the female accessions, cluster analysis on both morphological and biomolecular characters did not separate the Mediterranean from the Iranian-Caspian genotypes.

Barazani et al (2003) characterized two separated populations in Turkmenistan, Kepele and Agachli, seedlings from *P. vera* by RAPD technique. Cluster analysis was performed and divided two main groups according to their origins. The Agachli group was further divided into two subgroups indicating a close genetic relationship between the two populations. In their study small genetic distances and the high similarity values (0.58–1.00) revealed reasonable gene flow between Kepele and Agachli, which are 100 Km apart. Growing the accessions on the same plot, under similar conditions enabled the evaluation of genotypic differences.

Ahmad et al (2003) used 14 SSR locus to characterize American, Iranian, Turkish, and Syrian pistachio cultivars. Cluster analysis was performed and placed most of the Iranian samples in one group while the Syria samples were the most diverse and did not group in a single cluster.

Kafkas et al (2006a) characterized 69 pistachio cultivars and genotypes from Iran, Turkey, USA, Syria, Greece, Italy, Israel, Cyprus and Tunisia by AFLP, ISSR and RAPD analysis. Two main groups were identified. One group contained the cultivars originated from Iran, and the other group included cultivars originated from Turkey, Syria, Greece, Italy, Cyprus and Tunisia. The results showed distribution of the cultivars from Iranian-Caspian origin via southeastern Turkey to Syria, Mediterranean region of Europe and North Africa.

Kafkas et al (2006b) characterized 17 wild pistachio accessions collected from natural pistachio populations of Afghanistan by AFLP markers. A total of 228 AFLP fragments were obtained from eight primer combinations. The allele numbers for each primer combination ranged from 18 to 48. A total of 136 polymorphic bands divided 17 accessions based on their origins.

2.2. Phylogenenetic Analysis Of *Pistacia* Species By DNA Markers

Rovira et al (1995) reported the first genetic characterization study in *Pistacia* species who used six species (*P. atlantica* Desf., *P. integerrima* Stewart., *P. khinjuk* Stocks, *P. palaestina* Boiss. and *P. vera* L.) for 10 isoenzyme systems. Zymograms showed distinct banding patterns for the different species, which was useful for characterization analysis. The most polymorphic species were *P. atlantica, P. vera, P. palaestina,* whereas the least polymophic were *P. integerrima, P. khinjuk* and *P. terebinthus* L.

Louskas and Pontikis (1997) characterized four genotypes of *P. vera* and two wild *Pistacia* species, *P. terebinthus* and *P. lentiscus* L. with pollen isozyme patterns of nine different enzymes. The closer relationship was indicated between *P. vera* and *P. terebinthus* than *P. vera* and *P. lentiscus*.

Parfitt and Badenes (1997) classified nine *Pistacia* species by restriction of 3.2-kb region of variable chloroplast DNA. According to the authors, two groups of *Pistacia* were found. The first one is Lentiscus group included *P. mexicana*, *P. texana* Swingle, *P. weinmannifolia* Poiss. and *P. lentiscus*, and the second group was named as Terebinthus that contained *P. chinensis* Bunge., *P. integerrima*, *P. terebinthus, P. atlantica*, *P. khinjuk* and *P. vera*.

Kafkas and Perl-Treves (2001a) determined the relationships of common wild *Pistacia* species (*P. vera, P. atlantica, P. terebinthus* and *P. eurycarpa*) in Turkey by morphological data and RAPD analysis. A total of 40 wild *Pistacia* genotypes from different regions of Turkey were tested: 10 *P. eurycarpa* (locally identified as *P. khinjuk*) genotypes from Gaziantep and Siirt provinces and 20 *P. atlantica* and 10 *P. terebinthus* genotypes from Manisa, Adana and Aydın provinces. Two local *P. vera* varieties, Kirmizi and Siirt were added for comparison. Cluster analysis based on morphological data showed that *P. eurycarpa* is the closest species to *P. vera*, followed by *P. atlantica* and *P. terebinthus*. One hundred and thirty eight scorable bands were selected from ten polymorphic RAPD primers and used in cluster analysis. Four *Pistacia* species are clearly separated from each other and the closest relationship was determined between *P. atlantica* and *P. eurycarpa.*

Werner et al (2001) identified *P. saportae* Burnat. morphologically and by RAPD analysis as a hybrid between *P. lentiscus* and *P. terebinthus*. RAPD analysis produced several species-specific RAPD markers.

Kafkas and Perl-Treves (2002a) were examined phylogenetic relationships among nine *Pistacia* species by RAPD markers and were defined two main groups based on 242 bands. The first group included *P. vera*, *P. khinjuk*, *P. eurycarpa* Yalt., *P. atlantica*, and *P. integrrima*. The second group involved *P. palaestina*, *P. terebinthus*, *P. mexicana* and *P. lentiscus*. *P. vera* and *P. khinjuk* were highly similar, as were *P. palaestina* and *P. terebinthus*. The authors suggested that *P.*

palaestina is in fact the variety of *P. terebinthus* and *P.eurycarpa* is a distinct species from *P.atlantica*.

Katsiotis et al (2003) characterized five *Pistacia* species (*P. vera, P. palaestina, P. terebinthus, P. chinensis* and *P. lentiscus*) and one variety of *P. lentiscus* by AFLP and RAPD analysis. Two main groups were identified; one of them contained the evergreen species *P. lentiscus* and *P. lentiscus* var. Chia. The other group included deciduous species.

Golan-Goldhirsh et al (2004) studied taxonomic relationships of six Mediterranean *Pistacia* species (*P. vera, P. palaestina, P. terebinthus, P. atlantica, P. lentiscus* and *P. khinjuk*) by RAPD and AFLP markers. Totally, 1,285 bands were amplified and used for cluster analysis. *P. lentiscus* was in a separate group from other five *Pistacia* species.

Kafkas (2006a) was studied on phylogenetic analysis of 10 *Pistacia* species using AFLP technique. Six AFLP primer combinations were tested. Out of 275 bands, 254 were polymorphic bands (92.4%). The total numbers of polymorphic bands per primer pairs were found 45.8. The rate of polymorphic bands was calculated as approximately 90.5%. *P. atlantica, P. khinjuk, P. vera, P. mutica, P. integerrima* and UCB#1 (*P. atlantica* x *P. integerrima*) were placed in one group, and *P. lentiscus, P. palaestina, P. terebinthus* and *P. mexicana* were in the second group. *P. atlantica - P. mutica* and *P. terebinthus - P. palaestina* pairs were the closest species, therefore *P. mutica* were classified as *P. atlantica*, and *P. palaestina* were as *P. terebinthus.*

Vendramin et al (2009) characterized 82 accessions within *P. vera, P. terebinthus, P. mutica, P. khinjuk* and *P. integerrima* species using SSR markers. According to the cluster analysis *P. vera* was sperated from other *Pistacia* species. *P. vera* cultivars were separated in several different geneticly close subclusters, probably due to the limited genetic pool of cultivated materials. All the *P. terebinthus* were grouped in the same subcluster; *P. mutica* accessions were divided in three highly geneticly variated subclusters.

Salehi Shanjani et al (2009) characterized 216 *Pistacia* accessions including seven populations of three wild species (*P. khinjuk, P. vera,* and *P. atlantica* subsp. *kurdica*) and some important cultivars from Iran and other foreign cultivars using AFLP markers. Using unweight pair group method with arithmetic averaging (UPGMA), the analysis of molecular variance proved that there is high levels of genetic diversity within the Iranian cultivars which had clear separation from foreign cultivars. Among these species, *P. atlantica* subsp. *kurdica* showed the lowest degree of polymorphism with the lowest number of total bands as compared to the other species.

Karimi et al (2009) used 44 accessions of *P. vera*, *P. khinjuk*, *P. eurycarpa* and all subspecies of *P. atlantica* (*mutica*, *kurdica, atlantica,* and *cabulica*), three unknown genotypes and three accessions, proposed to be hybrid from *P. eurycarpa* × *P. atlantica* from Iran, Turkey, USA and Syria. A total of 475 fragments were produced by six AFLP primer combinations, with an average of 79.2 fragments per primer pair, of which 336 bands were polymorphic. Two main clusters were developed. *P. vera*, *P. eurycarpa*, *P. atlantica* (subsp. *atlantica*, *kurdica*, *mutica*, *cabulica*) and the hybrid genotypes located in the first main cluster. *P. khinjuk* accessions from Iran and USA localized in the second main cluster. The closest species to *P. vera* was *P. eurycarpa*. Cluster analysis revealed that *P. eurycarpa* is synonym for *P. atlantica* subsp. *kurdica* and should be considered distinct from *P. atlantica*; however, *P. atlantica* showed a closer genetic similarity to *P. eurycarpa* than the other species.

Pazouki et al (2010) used 22 foreign cultivars (*P. vera*) and 282 Iranian *Pistacia* genotypes (*Pistacia* spp.). The relationship between *Pistacia* species were tested using 10 SSR markers. The results showed that the genetic diversity within *P. vera* and *P. khinjuk* were much more higher than *P. atlantica* subsp. *kurdica*. Principal coordinate analysis showed a clear separation among the different *Pistacia* speices, as well as in the Iranian and foreign cultivars.

Karimi and Kafkas (2011) determined the genetic relationship among *Pistacia* species (*P. vera*, *P. khinjuk*, *P. eurycarpa*, *P. atlantica* subsp. *atlantica*, *P. atlantica* subsp. *mutica* and *P. atlantica* subsp. *cabulica*) using SAMPL technique. A total of 182 bands were obtained from six primer combitations, 128 were polymorphic with an average of 30.3 bands per primer pair. As a result, three groups were obtained, the first one included *P. vera*, and the second contained *P. khinjuk*, *P. eurycarpa*, *P. atlantica* and subspecies *cabulica* and *mutica*, with numerous amount of leaflets clustered in the third group. Cluster analysis made a separation between *P. atlantica* subspecies and *P. eurycarpa*.

Karimi et al (2012) used 16 accessions of *P. khinjuk* to reveal the genetic relationships and diversity among them. Leaf morphological characters together with RAPD technique were used. Using fifteen primers, a total of 146 fragments were produced with an average of 9.73 fragments per primer of which 129 were polymorphic. Three groups of genotypes were identifed: The first group were the genotypes which had round leaflets. The other two groups included genotypes with three lanceolate leaflets. According to these findings, high diversity occurs among genotypes of *P. khinjuk*, like in *P. vera*.

Talebi et al (2012) used 30 SRAP primer combinations (PCs) for characterization of 36 *Pistacia* accessions which were from Iran, Syria, Turkey and United States by SRAP technique. A total of 202 fragments were amplified by 11 PCs, which 168 (83%) of them were polymorphic. As a result of cluster analysis, the closest species of *P. vera* was *P. atlantica* followed by *P. khinjuk*. *P. vera* accessions were clustered in a separate group and wild “Sarakhs” accessions grouped together in a same cluster. The authors assumed that the domesticated genotypes of *P. vera* evolved from ‘Sarakhs’ accessions.

Iranjo et al (2016) used Randomly Amplified Polymorphic DNA (RAPD) for characterization of 50 wild accessions of *Pistacia* species (*P. vera*, *P. khinjuk*, *P. atlantica*, *P. mutica* and *P. eurycarpa*), and polymorphic information contents (PICs), and genetic similarities (GS) for RAPD markers were computed. A total of

146 (97.98 %) out of 149 fragments generated from 17 RAPD primers were polymorphic. Genetic similarities ranged from 0.30 to 0.86, and PIC values ranged from 0.80 to 0.99. According to the results, it was assumed to be a wide range of genetic diversity among the pistachio accessions as a useful tool for breeding programs for genetic conservation and cultivar development.

Abuduli et al (2016) used SSR, RAPD, ISSR, and ITS markers to determine the genetic variation and phylogenetic relationships in 12 males and 12 females of mastic tree (*P. lentiscus* L.) genotypes. As a result of scored alleles, the average number of alleles produced from SSR, RAPD, and ISSR primers were 5.7, 14, and 18, respectively. Bayesian clustering method was used for production of marker dataset and neighbor joining dendrograms were created. The results showed that the most powerful markers for differentiation of genotypes were ISSR and RAPD markers. The ISSR results indicated that male and female genotypes were distinctly separated from each other.

2.3. Cytological Studies In *Pistacia*

There are only a few chromosome count studies in *Pistacia*. The first study was reported by Zohary (1952) who found chromosome number of *P. vera*, *P. atlantica* and *P. lentiscus* species, 30, 28 and 24 respectively. The number of diploid chromosome for *P. vera* L. has been reported as 32 by Barghchi and Alderson (1989).

Özbek and Ayfer (1957) reported in his monographic observation of *Pistacia* species by studying the meiosis in pollen mother cell and found 2n=30 chromosome number in *P. vera*, *P. khinjuk*, *P. terebinthus*, and 2n=28 *in P. atlantica*.

Harandi and Ghaffari (2001) used Iranian species of *P. vera* for karyotype analysis. Meiosis in this species was regular and showed 15 bivalents at the first metaphase. The chromosome number was found as 2n=30. In the interphase, two

chromocenters were observed clearly, which were also visible in the prophase and metaphase.

Ghaffari and Harandi (2002) used *P. vera*, *P. khinjuk* and *P. atlantica* for karyotype analysis. The chromosome complement of *P. vera* was 2n=30, with four metacentric pairs, eight submetacentric pairs and three acrocentric pairs. One pair of metacentric chromosomes was heterochromatic in nature. The karyotype of *P. khinjuk* (2n=24) consisted of seven submetacentric pairs and five metacentric pairs, of which one pair was heterochromatic. Somatic chromosome counts in subspecies of *P. atlantica* including *cabulica*, *kurdica* and *mutica* gave a chromosome number of 2n=28, with three metacentric pairs, nine submetacentric pairs and two acrocentric pairs. In the three subspecies, one pair of metacentric chromosomes was heterochromatic. The comparison of all species and subspecies showed that two of the metacentric chromosomes could be candidates for the sex chromosome in this genus.

Ila et al (2003) used root tips of four *Pistacia* species (*P. vera*, *P. eurycarpa*, *P. atlantica*, *P. terebinthus*) to determine their chromosome numbers, and found 2n=30 in all four species. According to the results, chromosome numbers of different *Pistacia* species in the literature were fonud to be disputed and controversial due to the fact that poor chromosome counting protocols were used.

Ayaz and Namli (2009) used *P. vera* roots obtained *in vitro* from mature seeds for karyotype analysis. The chromosome number was found 2n=30 at *c*-metaphase of mitosis cell. Centromere type of all chromosomes were designated as median, submedian, subtelocentric, telocentric and total lengths of chromosome pairs were found between 35.4 and 5.97 micrometer (µm).

Al-Saghir et al (2014) used fluorescent microscope for karyotyping analysis in *Pistacia* species. Leaves from the herbarium specimens of *P. atlantica*, *P. khinjuk*, *P. lentiscus*, and *P. terebinthus* were used. The chromosome number was found as 2n=30 for all the analyzed species.

Sola-Campoy et al (2012) reported karyotyping of the dioecious pistachio tree (*P. vera* L.). The fluorescent in situ hybridization (FISH) with probes for 5S and 45S rDNAs, and two pistachio specific satellite DNAs (PIVE-40 and PIVE-180), together with DAPI-staining was used. PIVE-180 has a monomeric unit of 176–178 bp and high sequence homology between family members. PIVE-40 has a 43 bp consensus monomeric unit in which contained higher order repeats (HORs) of two units. In most chromosomes of *P. vera* genome which was highly heterochromatic, prominent DAPI positive blocks were detected. Although classifying chromosomes according to morphology was difficult, using a combination of FISH probes, 10 out of 15 pairs (2n = 30) could be distinguished by their unique banding patterns. The largest pair was designated as HC1, which was strongly heteropycnotic and showed massive enrichment in PIVE-40 repeats. HC1 chromosomes with differing PIVE-40 hybridization signal were divided in two types, type-I which had homozygotes, and type-II had both heterozygotes and homozygotes individuals. Suppression of homologous recombination at meiosis caused the differentiation between the two HC1 chromosomes which was due to reinforcement by the presence of PIVE-40 HORs and differences in PIVE-40 abundance. This would be compatible with a ZW sex-determination system in the pistachio tree.

2.4. Sex Determination In *Pistacia*

Sex chromosomes in land plants can change due to close linkage between the two sex determination genes with complementary dominance. This phenomenon is required for establishing stable dioecious populations, which are found in at least 48 species across 20 families. The sex chromosomes in hepatics, mosses, and gymnosperms are morphologically heteromorphic (Ming et al, 2011).

In evolution of sex chromosomes in dioecious species, two sex-determining genes are strictly linked on the same chromosome with complementary dominance (Charlesworth et al, 1978). The suppression of recombination between the two sex

determination genes is a crucial event in sex chromosome evolution. In the first step of sex chromosome evolution, mutations are happened in the entity of male and female which leades to the development of unisexual reproductive structures (Ming et al, 2011). Although the flower primordia in the buds of female and male pistachios are simultaneously initiated in trees of both sexes, but the diffrences are becoming apparent as the trees growth and developes. In opposite sex plants, the development of sex organs is suppressed at the primordial stage (Hormaza et al, 1996).

Due to suppression of recombination in sex chromosomes, sex determination is based on the presence of either homomorphic, (e.g., in *Carica papaya*), or heteromorphic sex chromosomes (e.g., in *Silene latifolia*) in some plant species (Liu et al, 2004, Mariotti et al, 2006). As a result of the suppressed recombination, repetitive sequences, inversions, deletions, or translocations leads accumulate in different plant species (Liu et al, 2004, Lengerova et al, 2004, Na et al, 2014).

In the cultivar-breeding programs it is aimed to reduce the cost by marker assisted selection (MAS), but as pistachio is a dioecious species, then eliminating of males can be an effective strategy. Hormaza et al (1994) was the first on finding sex marker in pistachio by RAPD technique. The authors used bulk segregant analysis and a segregating population in *P. vera*. Two bulks of DNA contained one from males and one from females in each cross; for the 'Lassen x Peters' cross fourteen males and 10 females and for the 'Kerman x Peters' cross 9 males and 12 females were used. Consequently, a single sex marker ($OPO08_{945}$) linked to sex determination in pistachio was found.

Kafkas et al (2001b) specified RAPD markers that associated to sex character in two major wild species in Turkey; *P.atlantica* and *P.eurycarpa*. These species are used as rootstocks for *P. vera*. By screening 472 RAPD primers, one sex marker ($BC156_{1300}$) in *P.urycarpa*, one ($OPAK09_{850}$) in *P.atlantica* and none in *P.terebinthus* was found.

Yakubov et al (2005) developed a female DNA marker in *P. vera* using combined sequence characterized amplified regions (SCAR) primers and touchdown-PCR methods. The primer $OPO08_{945}$ amplified a 905-bp fragment in all female trees, but also in several male trees. SCAR primers were designed based on the RAPD female marker and amplified a 905-bp female and a 909-bp male fragment which verified on 54 different pistachio genotypes. On the basis of a polymorphic locus, a second internal set of SCAR primers were designed, in combination with touchdown-PCR technique. All females amplified a specific 297-bp product, while males had no amplification product. Therefore, new set of SCAR primer pairs with touchdown PCR were suggested to determine females in a breeding program or in a germplasm collections in *P. vera*.

Esfandiyari et al (2012) used *P. atlantica* subsp. *mutica*, *P. khinjuk* and *P. vera* subsp. *Sarakhs* to find sex marker for wild species. Leaves were collected from both male and female plants of each species for DNA extraction. Thirty RAPD primers and one pair of SCAR primer were tested to identify a marker closely linked to the sex loci in wild *Pistacia* species. Among the RAPD primers, only BC_{1200} was succesfully amplified a specific sex band present in female plants. Among all individual samples, a fragment of approximately 300 bp was amplified in female trees but not in male ones.

Sun et al (2014) aimed to find sex markers in *P. chinensis* Bunge by RAPD and ISSR techniques. Among 21 random-amplified polymorphic DNA primers and 20 inter-simple sequence repeat primers, two sex-specific primers (S1 and S281) were identified which were able to amplify female-specific fragments of 473 and 1242 bp, respectively. However, only one fragment (FS281) was converted successfully into a sequence-characterized amplified region (SCAR) marker. 636 bp fragment was amplified by the SCAR primer pairs in all females while it was absent in all male samples in annealing temperature of 64°C.

Kafkas et al (2015) applied Restriction site-associated DNA (RAD) markers to elucidate the mechanism of sex determination in pistachio and also to

identify markers related to sex in pistachio. As a material of RAD sequencing in two lanes, F1 population from a *P vera* Siirt × Bağyolu cross of eight female and eight male and also their parents, were used to sequence by Hi-Seq 2000 platform. Out of 37.7 Gb of sequences, 33,757 polymorphic single nucleotide polymorphism (SNP) loci were identified between the parents. Thirty-eight of sequences from 28 RAD reads were detected as putative sex-associated loci in pistachio. Among 13 tested markers, eghit of them could determine sex with 100% accuracy in pistachio. According to the SNaPshot analysis, five of the loci could not seperated in F1 progenies were discarded. Further analysis were done using the remaining eight SNP loci from seven reads. Therefore, seven pairs of SNP flanking primers were used in the HRM analysis which another SNP genotyping method for rapid and cost-effective analysis of genetic variation. Among the seven tested SNP loci, four (SNP-PIS-133396, SNP-PIS-136404, SNP-PIS-167992, SNP-PIS-174431) could distinguish sex in all 166 tested plants. Whereas HRM analysis was not able to determine sex in the remaining three SNP loci. As a result, four loci were identified to be a proper tool for marker-assisted selection (MAS) in breeding programs. Because of the female heterogamety in all candidate SNP loci, they reported for the first time that pistachio has a ZZ/ZW sex determination system.

Khodaeiaminjan et al (2017a) tested 25 remaining sex-associated SNP markers from the study by Kafkas et al. (2015). The authors extented the RAD sequences to allow primer design for validation by SNaPshot analysis. Of the 25 markers, the authors found 9 more SNP markers linked to sex in pistachio. All the markers were heterozygote in females and homozygote in males, supporting ZW/ZZ sex determination system in pistachio.

2.5. Development Of SSR Markers In *Pistacia* By Classical Methods

The first SSR markers in pistachio were developed from 'Kerman' pistachio (*P. vera*) cultivar. Di-nucleotide (CT and CA) and tri-nucleotide (CTT) enriched genomic DNA libraries were firstly constructed. Eighty-nine clones randomly

selected from 295 clones for 'CT/GA' enriched library. About 57 clones (64%) contained CT repeats. Sixty-two clones out of 250 clones, randomly selected from 'CA/GT' enriched library. Thirty-seven (59%) clones contained CA repeats. Of the total 151 clones, 67 (44%) clones had sufficient flanking sequence for primer design. Thirty-three clones were selected randomly for 'CTT/GAA' enriched library. Eight (24%) clones had sufficient flanking sequence for primer design. Out of 25 designed primer pairs, 14 pairs were amplified clearly and produced an easily explicable PCR product in all the commercially important American, Iranian, Turkish and Syrian pistachio cultivars (Ahmad et al, 2003).

The second SSR development study was published by Albaladejo et al (2008) who developed eight polymorphic nuclear microsatellite markers for *P. lentiscus* by an enriched genomic library method. Forty-two individuals from two populations sampled in Southern Spain were used for characterization of eight loci. Total of 59 alleles were detected, ranging from three to 13 per locus. Expected heterozygosity ranged from 0.139 to 0.895.

Arabnezhad et al (2011) constructed two enriched DNA libraries with dinucleotide (AG) and trinucleotide (ATG) microsatellite motifs in *P. khinjuk.* Colony PCR technique were used to screen of clones and 44 clones were sequenced. Twenty seven primer pairs were designed from flanking regions of the repeats Designed primer pairs showed successful cross-species amplification within the genus *Pistacia*. According to the minimum evolution clustering algorithm in dendrogram on the basis *P. vera* has closer relationships with *P. khinjuk*, than with *P. integerrima*, *P. palaestina*, *P. atlantica* and *P. mutica*. Also the wild Sarakhs pistachio from the rest of *P. vera* cultivar suggesting of the domesticated genotypes of *P. vera* which are evolved from *P. vera* var. Sarakhs.

Chen et al (2011) developed SSRs from gnomic DNA of *P. weinmannifolia*. A total of 147 sequences contained SSRs from 205 sequenced clones, and 94 allowed primer design with sufficient flanking regions. Fourteen polymorphic microsatellite loci screened on 12 individuals from two wild *P.*

weinmannifolia populations and had an average of 4.1 alleles per locus. *P. chinensis* aand *P. mexicana* were used to test the transferability of the 14 primer pairs. Nine (64.2%) loci in *P. chinensis* and four (28.6%) loci in *P. mexicana* were successfully transferable.

Zaloglu et al (2015) developed SSR markers from *P. vera* cv Siirt. Genomic libraries enriched with the repeats of 'CA', 'GA', and 'AAC' and 'AAG' were constructed. From each library fifty-seven clones were selected and a total of 228 clones were sequenced. Out of 84 designed primer pairs, 59 generated (70.2%) bands and 47 were polymorphic in the characterization of seven diverse pistachio cultivars. The number of alleles among 47 polymorphic loci ranged from two to nine with an average of 3.6. Among the four libraries in conjunction with polymorphism, number of alleles and informativeness, the GA-enriched library was the best, while the AAC library was the worst. Di-nucleotide-enriched libraries was obtained higher number SSRs than tri-nucleotide libraries. Fifty-nine SSR primer pairs were tested in eight *Pistacia* species. Fifty-four out of 59 tested SSR primer pairs were transferable to at least one *Pistacia* species.

Topçu et al (2016) developed simple sequence repeat (SSR) markers from *P. vera*. Genomic libraries were constructed from enriched dinucleotide (GA)n and trinucleotide (AAG)n microsatellite motifs. From each library, 192 clones were sequenced and 135 primer pairs were designed. From designed primer pairs 110 PCR products were generated. Forty-six polymorphic loci from the GA library and 18 polymorphic loci from the AAG library were produced by testing of 12 pistachio cultivars. From the polymorphic loci, 264 alleles were produced with an average of 4.13 alleles per locus. The observed (Ho) and expected heterozygosity (He) values were 0.52 and 0.56, respectively while polymorphism information content (PIC) was 0.51. One hundred out of 110 SSR primer pairs were transferable to at least one of the tested ten *Pistacia* species.

2.6. Next-Generatıon Sequencing (NGS) Technology For SSR Marker Development

The sequencing of DNA molecules began in the 1970s with development of the Maxam-Gilbert method, and then the Sanger method was developed. Two different labelling protocols were used to perform automated DNA sequencing. One of them is known as dye-labelled primer chemistry and the other is known as dye-labelled terminator chemistry (Lilian et al, 2002).

The first automatic sequencing machine introduced by Applied Biosystems (namely ABI370) in 1987, adopting capillary electrophoresis which made the sequencing faster and more accurate. Emerged in 1998, software using the capillary sequencing machineries and associated the automatic sequencing tools and Sanger sequencing technology became the key tools for the genome projects (Collins et al, 2003).

NGS are different from the Sanger method in aspects of enormously parallel analysis, high quantity, and condensed price. Although NGS makes genome sequences handy, the followed data analysis and biological explanations are still the bottle-neck in understanding genomes. Costs and time were reduced, in Compared with the traditional development of microsatellites from a genomic library as provided by many commercial suppliers (Schnable et al, 2009).

An easy procedure for SSR development or microsatellite loci is the application of NGS technologies for genetic research. Microsatellite markers are one of the most informative and versatile DNA-based markers. These markers were used in large-scale sequencing studies resulting in complete genomic sequences of plants ranging from the 125 Mb *Arabidopsis thaliana* to the 2.5 Gb *Zea mays* (Schnable et al, 2009).

Csencsics et al (2010) developed SSR primers in dwarf bulrush (*Typha minima* Funck ex Hoppe) by NGS technology. Three hundred and seven sequences were detected with tandem repeats: 43 di-, 163 tri-, and 101 tetra-nucleotide microsatellites with at least 8, 10, and 6 repeats, respectively. One-hundred of

detected sequences were selected to design primer which had suitable annealing sites. Seventeen polymorphic microsatellite markers with perfect repeats were choosed to test in 20 individuals. Two to seven alleles were detected per locus. Expected and observed heterozygosities were calculated 0.05-0.76 and 0.07-1.00, respectively.

Iorizzo et al (2011) developed SSR primers in carrot by *de novo* assembly. A total of 58.751 contigs and singletons were produced from four genetic backgrounds. Over the half of assembled sequences were annotated allowing detection of transposable elements and new carrot anthocyanin genes. At a depth of coverage of 20×, One-hundred and fourteen computationally polymorphic SSRs were designated. Amplification of a product in about 90% of tested SSR primers was observed. Out of verified SSR markers, 84% were polymorphic.

Zhu et al (2012) used American cranberry (*Vaccinium macrocarpon* Ait.) cultivar 'HyRed' to perform low-coverage whole genome shotgun sequencing. By using of Roche 454 sequencing technology, the sequences which were covered 266.3 Mb of the estimated 540-590 Mb in cranberry genome, were obtained. A total of 107,244 SSR loci were identified with an total density across the genome of 403 SSR/Mb. The most frequent motif in cranberry was the AG repeat which accounting for 35% of all SSRs and together with AAG and AAAT accounted for 46% of all loci discovered. Contig sequence data were used to design primer pairs and finally ninety-six primer pairs containing perfect SSR repeats were designed. In the genetic diversity of 25 cranberry genotypes, forty-eight polymorphic SSR loci with 2-15 alleles per locus were detected.

Park et al (2013) developed and characterized genomic SSR markers using oriental melon (*Cucumis melo* var. makuwa) from the preliminary Illumina read assembly. The most abundant type of markers was mononucleotide motifs followed by di-, tri-, tetra-, and pentanucleotide. AT, followed by AG and AC motif types, were the most abundant dinucleotide repeats and AAT motif was the most frequent in trinucleotide repeats. Two hundred and thirty six out of 370 designed primer

pairs were tested in Gotgam chamoe (one of the major landraces; KM) and SW3 (the breeding parent), observing 93 % polymorphism between KM and SW3. Seven selected SSR markers were used in KM, SW3, and four chamoe cultivars. A total of 17 alleles with 2–3 alleles per locus were identified in the first report of genomic resources of Korean landraces.

Celik et al (2014) sequenced genomic DNA of opium poppy (***Papaver somniferum*** L.) by pyrosequencing technology to develop simple sequence repeats (SSRs). A total of 1.244.412 sequence reads were obtained covering 474 Mb. Fifty two percent of reads were assembled into 166,724 contigs which represented 105 Mb of the opium poppy genome. A total of 23.283 nonredundant SSRs out of 18.944 contigs were identified (11.3 % of total contigs). The most abundant SSR repeats were tri-nucleotide and tetra-nucleotide. AT-rich repeats were also most common repeats in tetra-, penta- and hexanucleotide SSRs. A total of 23.126 primer pairs (98.7 % of total SSRs) were designed to amplify SSRs. Due to determination of polymorphism and transferability, fifty-three genomic SSR markers were tested in 37 opium poppy accessions and seven ***Papaver*** species. The average of intra-specific polymorphism information content (PIC) values of the genomic SSR markers were 0.17, while the interspecific average PIC value was slightly higher, 0.19.

Xiao et al (2015) aimed to characterize simple sequence repeats (SSRs) in the Chinese jujube (***Ziziphus jujuba***) genome. A total of 436,676 SSR loci were identified with an average distance of 0.93 Kb. The most frequent SSR repeat motifs were mononucleotide (64.87%), followed by dinucleotide (24.40%) and trinucleotide (8.74%). A/T motif was the most common mononucleotide repeats, whereas AT/TA motif was the most frequent dinucleotide repeat. A total of 725 primer pairs were selected randomly from 1,000 primer pairs and 511 were polymorphic in six cultivars. The transferability of jujube SSR primers proved that 35 from 64 randomly selected SSRs could be transferred across the family.

Hou et al (2016) developed SSR markers in tartary buckwheat for analysis of genetic diversity. The genome survey technology generated 21.9 Gb raw sequence reads which were assembled into 348.34 Mb genome sequences involved 204,340 contigs. According to K-mer analysis, the genome size was assessed about 497 Mb. In total, 24,505 SSR motifs were identified and the most frequent motif types were dinucleotide (67.14 %) and tri-nucleotide (26.05 %) repeats. AT/AT motif was the most common repeat motifs, accounting for 78.60 % of di-nucleotide repeat motifs. SSR screening of 64 accessions produced 49.7 effective number of allele from a total of 63 allele. SSR data were used to perform of population genetic structure analysis and 64 accessions of tartary buckwheat were clustered into two separate subgroups.

Khodaeiaminjan et al (2017b) designed 750 polymorphic SSR primer pairs *in silico* in pistachio by next generation sequencing (Illumina) of three *P. vera* (Siirt, Ohadi and Bağyolu) cultivars and one monoecious *P. atlantica* genotype (Pa-18) at 10x coverage. Testing the primer pairs in 18 *P. vera* cultivars and 6 *P. atlantica* genotypes by SSR-PCR analysis produced 625 polymophic loci from 618 primer pairs. A total of 3,947 alleles were obtained from 625 loci with an average of 6.2 allele per locus.

2.7. Construction Of Genetic Linkage Maps In *Pistacia*

There are two genetic linkage maping studies in pistachio. Türkeli and Kafkas (2013) used 16 ISSR primers, 27 SRAP and 27 AFLP primer combinations to construct the genetic linkage map of pistachio. ‘Double pseudo-testcross’ stategy were used to construct two separate genetic linkage maps. Ninty-two F1 individuals in inter-specific population of a cross between ‘Siirt’ (*P. vera*) cultivar and monoecious ‘Pa-18’ (*P. atlantica*) genotype were used. A total length of ‘Siirt’ maps were 1237.3 cM with 17 linkage groups and a mean marker density was calculated 7.50 cM. ‘Pa-18’ map with 19 linkage groups covering 1337.5 cM and

an average marker density was 8.57 cM. Some of LGs in parental maps were not well saturated; in ‘Siirt’ map with four and in ‘Pa-18’ map with 5 minor LGs.

Recently, Khodaeiaminjan et al (2017b) constructed SSR-based genetic linkage map of pistachio. One-hundred and twenty-two F1 progenies derived from a cross between ‘Siirt’ cultivar and ‘Bağyolu’ genotype were used. A total of 1,027 SSR markers consisting of 625 novel loci and 402 previously developed from other studies were used to construct the first reference map in pistachio. A total of 385 SSR markers were mapped in intraspecific population. The consensus map had 1511.2 cM length and average distance between markers was 3.9 cM with a 0.25 marker density. In ‘Siirt’ genetic map, a total of 318 SSR markers were mapped with a 0.22 marker density. ‘Siirt’ genetic map had 15 linkage groups spanning 1429.2 cM length with 4.5 cM distance between the markers. A total of 341 markers were mapped in ‘Bağyolu’ genotype with a 0.22 marker density. ‘Bağyolu’ genetic map had 1453.4 cM length with 4.2 cM distance between the markers. The authors also used common markers to construct the first SSR based reference map pistachio, and 273 common markers were mapped along with 15 linkage groups with a total length of 1,365 cM.

3. MATERIAL AND METHOD

3.1. Plant Materials And DNA Extraction

For the genome survey study, DNA from the *Pistacia vera* cv. Siirt was sequenced using an Illumina (Hi-Seq 2000) next generation sequencing platform. Library construction for the short-paired libraries was conducted according to the manufacturer's protocol (http://www.illumina.com). Twenty-four (Kerman-USA, Kerman-Spain, Peters, Kallehghouchi, Sultani, Kaska, Uzun, Kirmizi, Barak Yildizi, Uygur, Atli, Tekin, Ajamy, Aeginea, Halebi, Ashoury, M3, M7, Silvana, Sirora, Red Aleppo, Siirt, Ohadi, and Bağyolu) *P. vera* cultivars were used to test SSR markers for polymorphism and to assess genetic diversity. The origins of the cultivars were previously described by Kafkas et al (2006a). Fresh leaves of 20 wild *Pistacia* genotypes (four genotypes from each five wild *Pistacia* species) belonging to *Pistacia atlantica*, *Pistacia integerrima*, *Pistacia terebinthus*, *Pistacia chinensis,* and *Pistacia lentiscus* were also collected to test transferability of SSR markers across *Pistacia* species and to analyze genetic diversity within each species. Fresh leaves of all the samples were collected from germplasm collections of Çukurova University in Adana and Pistachio Research Institute in Gaziantep.

An inter-specific F1 population derived from *P. vera* cv Siirt x *P. atlantica* Pa-18 monoecious genotype was used to construct genetic linkage map by SSR markers. A total of 126 F1 progenies were used in linkage mapping. One-hundred F1 progenies were obtained in 2004 from a TUBITAK project (TOATAG 2721) and the remaining were in 2014 in Gaziantep Pistachio Research Institute (Kafkas, 2003).

Siirt cultivar is a Turkish domestic variety of *P. vera*. It has ovoid nut shape and is one of the best Turkish varieties in terms of splitting rate (92%), yield and nut size (Ak and Acar, 2001). It has greenish, long and wide leaves with 1-2 numbers of leaflets. It has 15-20 cm leaf length with a very light young shoot color. Nutshell colour is ivory. Nut length is 19.91 milimeter (mm) and nut width 11.55

mm and nut thichness 11.02 mm. Siirt cultivar has slightly dehiscent hulls and yellowish hull colour with regard to splitting rate (92%; Gökçe and Akçay 1993).

Monoecious *P. atlantica* 'Pa-18': genotype is fully monoecious which was reported by Kafkas et al (2000), and used in the breeding program (Kafkas et al, 2003). Pa-18 genotype has a typical *P. atlantica* morphological characters: leaves are dark green, shorter and narrower than Siirt cultivar; one year shoots are brownish and has 3-4 number of leaflets.

Furthermore, 12 female (Siirt, Kerman ABD, Ohadi, Sultani, Uzun, Tekin, Kallehghouchi, 12-2, 14-107, 16-20, Sell7, Sell9) and 12 male (Bağyolu, Kaska, Uygur, Atli, Erkek7, 26-63, 16-32, 21-125, 25-90, 9-64, Peters, M7) *P. vera* cultivars and genotypes were used verified candidate SSR sex markers in this study.

Genomic DNA was extracted using the CTAB protocol (Doyle and Doyle, 1990) with minor modifications as described by Kafkas et al (2006a). Leaf tissue (1 g) was used for each sample. The leaves of each sample were then ground to a fine powder in liquid nitrogen, transferred to 15ml tube (buffer extraction: 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, %2 CTAB, %2 PVP, *B*-mercaptoethanol, %0.1 $Na_2S_2O_5$) and incubated at 65°C for 60 minutes. The samples were then mixed with 6 ml chloroform-isoamyl alcohol (24:1) and centrifuged at 14,000 rpm for 15 minutes. The aqueous layer was recovered and mixed with 2/3 volume of cold isopropanol to precipitate the DNA, then stand in -20°C for 24 hours and isopropanol was poured of and the precipitated nucleic acid washed with 70% ethanol. The isolated DNA was dried and resuspended in 50 µl water (Thermo Scientific, HyClone - Molecular Biology Grade Water, Cat No; SH30538.03). Quality and quantity of extracted genomic DNAs were determined by agarose gel electrophoresis and Qubit Fluorimeter (Invitrogen), respectively. The isolated DNAs were diluted to approximately 10 ng/µL and subjected to polymerase chain reaction (PCR) amplifications.

3.2. Genome Survey And Microsatellite Identification

For the genome survey study, 26.77 Gb clean data were generated after removing low quality reads from libraries. 18.72 Gb data was from a 250-bp library with 150-bp pair-end (PE) reads and 8.05 Gb data was from a 500-bp library with 90-bp PE reads. All data were used to perform K-mer analysis. Based on the results of the K-mer analysis, information on peak depth and the number of 17-mers was obtained and used to estimate the size of the genome, repetitive and heterozygosity. Assembly was performed using SOAPdenovo2 software (Luo et al, 2012) and the GC depth distribution was determined by SOAPaligner (Li et al, 2008).

SSR loci were searched using SSRIT (Temnykh et al, 2001) software. The search parameters were set for the detection of di-, tri-, tetra-, penta-, and hexanucleotide SSR motifs with a minimum of 6, 5, 4, 4, and 4 repeats, respectively. The SSR loci were subjected to primer design using Primer 3 web based software (Rozen and Skaletsky, 1999) with the standard parameters.

3.3. SSR Primer Design And Selection

A total of 950 randomly selected primer pairs were synthesized and used for SSR marker development. PCR and capillary electrophoresis were performed to initially screen SSR primer pairs for polymorphism using two *P. vera* cultivars (Siirt and Bağyolu) and one monoecious *P. atlantica* genotype (Pa-18), which are parents in the monoecious cultivar breeding program (Kafkas et al, 2003). Then, SSR primer pairs were selected for further studies based on their polymorphism and ease of scoring.

SSR markers were prefixed with CUPVSiirt; CU denotes Cukurova University and PVSiirt denotes the *Pistacia vera* cv. Siirt, from which the SSRs were isolated. Following digits were obtained from the SSR number, x and y were used to identify different SSR loci produced by the same primer pair.

3.4. SSRs In The Literature

There are several studies on SSR development in the literature, and those SSRs were also used in this study. SSR primer pairs screened and used in this study are summerized with their origins in Table 3.1.

Table 3.1. Summary of recently developed SSR primers in different *Pistacia* genus which used in this study.

Reference	Origin	Acronyms	Number of SSRs
Ahmad et al. (2003)	*P. vera* cv 'Kerman'	Pmts	14
Albaladejo et al. (2008)	*P. lentiscus*	Pislen	8
Arabnezhad et al.(2011)	*P. khinjuk*	PKAG	27
Chen et al. (2012)	*P. weinmannifolia*	PW	14
Zaloglu et al.(2015)	*P. vera* cv 'Siirt'	CUPV	59
Topçu et al. (2016)	*P. vera* cv 'Siirt'	CUPV	110
Khodaeıamınjan et al. (2017a)	NCBI database	PVGH, PAEF, PVJQ, PVJN, PVDQ, PVFJ	170
Khodaeıamınjan et al. (2017b)	*P. vera* .cvs Siirt, Ohadi and Bağyolu, and *P. atlantica*	CUP	618
Total			1,020

3.5. SSR -PCR Conditions

SSR-PCR was carried out using a three primer strategy according to the method described by Schuelke (Scheulke, 2000) with some modifications (Topcu et al, 2016). PCR was performed in a total volume of 12.5 µL containing 20 ng DNA, 75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 2.0 mM $MgCl_2$, 0.01% Tween 20, 200 µM each dNTP, 10 nM M13 tailed forward primer at the 5 end, 200 nM reverse primer, 200 nM universal M13 tail primer (5 TGTAAAACGACGGCCAGT-3) labeled with one of FAM, VIC, NED, or PET dyes, and 0.6 *Dream Taq* DNA polymerase (Thermo Fisher Scientific, EP0705).

Amplification was performed in two steps as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 45 s, 58°C for 45 s, and 72°C for 60 s. The second step included 10 cycles at 94°C for 45 s, 58°C for 45 s and 72°C for 60 s, and a final extension at 72°C for 10 min. When the PCR was completed, the reactions were subjected to denaturation for capillary electrophoresis in an ABI 3130xl genetic analyzer [Applied Biosystems (ABI) Inc., Tokyo-Japan] using a 36-cm capillary array with POP7 as the matrix (ABI). Samples were fully denatured by mixing 0.5 mL of the amplified product with 0.2 mL of the size standard and 9.8 mL formamide. The fragments were resolved using ABI data collection software 3.0, and SSR fragment analysis was performed with GeneScan Analysis Software 4.0 (ABI).

3.6. Amplification Of Sex-Linked Markers In Siirt X Pa-18 F1 Population

The female sex-specific SCAR marker PVF1 developed by Yakubov et al (2005) and 16 sex-linked SNP markers developed by Kafkas et al (2015) and Khodaeiaminjan et al (2017a) in *Pistacia vera* L. were analyzed in the population.

The PCR reaction and condition of the SCAR marker (PVF1) were done according to Yakubov et al (2005). Amplified DNA fragments were resolved in 1.5 % TBE agarose gel, stained with ethidium bromide, and visualized under ultra violet (UV) light. The results showed a single band could be amplified in some of F1 population and was scored as present/absent in each individual.

SNaPshot technique was used in the analysis of 16 SNP sex-linked markers. Firstly, SNP flanking primers were applied to amplify PCR in which a reaction volume of 25 µL containing, 75 mM Tris–HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1% Tween 20, 100 µM each of dATP, dTTP, dGTP, and dCTP, 0.2 µM each of reverse and forward primers, 1.0 unit Hotstart Taq DNA polymerase, and 20 ng of genomic DNA were used. Thermal cycler conditions were: 2 min at 94°C for pre-denaturation; 35 cycles of 45 s at 94°C for

denaturation, 45 s at 50°C for annealing, and 1 min at 72°C for extension; followed by a final incubation of 5 min at 72°C. In order to remove excess dNTPs and primers, the treatment of 5 μL PCR product was done by 1 unit of shrimp alkaline phosphatase and 10 units of exonuclease I (Thermo Scientific, Vilnius-Lithuania) at 37°C for 30 min following by incubation for 10 min at 85°C for inhibition and inactivation (Kafkas et al, 2015)

SNaPshot analysis was performed using an Applied Biosystems SNaPshot Multiplex Kit [Applied Biosystems (ABI) Inc., UK] according to manufacturer protocol with minor modifications. Reaction volume was 10 μL, containing 5 μL SNaPshot Ready Multiplex Ready Reaction Mix, 0.5 μM single-base extension primer, and 1 μL shrimp alkaline phosphatase/exonuclease-treated PCR product. The thermal cycler conditions were 10 s at 96°C; 30 cycles of 10s at 96°C, 5 s at 50°C, and 30s at 60°C; followed by 2 min at 60°C. One unit shrimp alkaline phosphatase were used to treat labeled extension products. Then 9.8 μL Hi-Di formamide and 0.2 μL GeneScan-120 LIZ Size Standard [Applied Biosystems (ABI) Inc., Foster City, CA] were mixed with one microliter of diluted extension product. Denaturation step was done at 95°C for 5 min following electrophoresis using an ABI PRISM 3130xl Genetic Analyzer [Applied Biosystems (ABI) Inc., Tokyo-Japan] with a 36-cm-length capillary array and POP-7 polymer [Applied Biosystems (ABI) Inc., Foster City, CA]. GeneMapper 4.0 software (ABI).was used for data analysis (Kafkas et al, 2015).

3.7. Phenotypic Data Collection From Siirt X Pa-18 F1 Population

Most of the F1 progenies in Siirt x Pa-18 F1 population are still in juvenility stage, therefore leaf and shoot phenotypic traits were studied in the population for QTL (Quantitative Trait Loci) analysis. Measurements or observations on leaf length (LL), leaf width (LW), number of leaflet (NLL), young (one-year) shoot color (YSC), and leaf color (LC) were performed in the F1 progenies in 2015 and 2016 growing seasons. Leaf length and leaf width of ten

representative mature leaves of each individual were measured by ruler and the means were calculated to represent the traits of LL and LW. Ten representive leaflets for each tree individual were counted for the number of leaflets (NLL). The shoot color was done by observation on the F1 progenies. HunterLab instrument was used for measuring leaf color in ten replicates for each progeny and the means were calculated to represent the traits of LC. The L value represents the brightness of the leaves and +a value represents redness, -a value was greenness, and + b value represents yellow and -b showed blue (Gould, 1977). Chroma (c) value showed the vitality and maturity of the leaf color and calculated by using a and b values. A statistical analysis of phenotypic data was done using Microsoft Excel 2010. The correlation between measurements and distribution frequency of LL, LW, NLF, YSC and LC traits were calculated during 2015 and 2016 growing seasons.

3.8. Data Analysis

3.8.1. Genetic Diversity Analysis

A total of 204 SSR primer pairs were selected in the initial screening and they were used to evaluate the genetic diversity of 24 ***P. vera*** cultivars. The SSR loci were also tested to determine their genetic diversity and transferability to ***P. atlantica***, ***P. terebinthus***, ***P. integerrima***, ***P. chinensis***, and ***P. lentiscus*** species. Transferability of the SSR markers was calculated for each ***Pistacia*** species by comparing the number of amplified loci with the total number of loci analyzed. Number of alleles (***Na***), number of effective alleles (***Ne***), observed (***Ho***), and expected (***He***) heterozygosity were calculated using GenAlEx version 6.5 (Peakall et al, 2012). The polymorphism information content (***PIC***) of each locus was calculated using PowerMarker software version 3.25 (Liu et al, 2005). A dendrogram was obtained using NTSYSpc v2.22 (Rohlf, 2011) software by unweighted pair-group method with arithmetic averages (UPGMA). STRUCTURE 2.3.4 software (Pritchard et al, 2000) was also used to determine the number of

populations and for the construction of the population structure. The burn-in period and Markov chain Monte Carlo (MCMC) were set at 50,000 and 500,000, respectively. The average value of ln likelihood when K changed from 1 to 10 was calculated according to their genetic similarity, and each run was replicated five times to ensure consistency of results.

3.8.2. Genotypic Data Analysis For Linkage Map Construction

SSR and SNP markers were screened for segregation using parents and 6 F1 DNAs, and their segregation patterns were determined. Co-dominant scoring was performed in all the segregated markers. When both parents are heterozygous (abxcd, efxeg and hkxhk) they were called as common markers, and when one of the parent is heterozygous (nnxnp and lmxll) they were called as parental markers (Table 3.2). Furthermore, null alleles in some SSR markers were scored as present and absent allele which help to determine the null allele segregation (lmx00, 00xnp, abxc0, a0xcd Table 3.2). The SCAR marker was scored as dominant marker in the agarose gel.

Table 3.2. Segregation types of co-dominant SSR markers

Segregation Types	Segregation	Female parent		Male parent	
		Allele 1	Allele 2	Allele 1	Allele 2
abxcd	(1:1:1:1)	a	b	c	d
abxc0	(1:1:1:1)	a	b	c	0
a0xcd	(1:1:1:1)	a	0	c	d
efxeg	(1:1:1:1)	e	f	e	g
hkxhk	(1:2:1)	h	k	h	k
lmxll	(1:1)	l	m	l	l
lmx00	(1:1)	l	m	0	0
nnxnp	(1:1)	n	n	n	p
00xnp	(1:1)	0	0	n	p

3.8.3. Construction Of Genetic Linkage Maps And QTL Analysis

Linkage maps were constructed by using the double pseudo-testcross mapping strategy (Grattapaglia and Sederoff, 1994) for each parent independently. Furthermore all segregating markers were used to construct a consensus map . Besides parents' and consensus map, a reference map with common markers was also built.

Linkage analysis were performed using JoinMap version 4.1 (Van Ooijen, 2011). The chi-square (X^2) test for each locus was applied to test for goodness of fit the segregating markers of Mendelian ratios hypothesis (Van Ooijen, 2011). The log of odds (LOD) scores based on G^2 statistics 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 difficulty to determine their linkage phases. Markers also removed when their distorted segregation conflicted with the segregation pattern of neighboring markers.

To determine the linkage threshold of markers, a LOD score of 5.0 was set initially for all maps in the same linkage group by standard JoinMap parameter settings. In order to expressing recombination frequencies as linkage distance in centiMorgans (cM) of maps, Kosambi's mapping function was used (Kosambi, 1994). The regression mapping algorithm was used for map construction. The presentation of the maps was done using MapChart 2.2 for Windows (Voorrips, 2002) and the gaps of $\geq$20 cM were represented by black segments.

For the mapped distorted markers, allelic and zygotic SD tests were performed using the methods described by Li et al (2011). The observed allelic ratio of each heterozygous parent was calculated and compared to the expected 1:1 ratio by a $\chi 2$ test for an allelic SD test. The expected genotypic ratio was estimated based on the allele frequency in the F1 population, and then a $\chi 2$ test was performed to test the deviation of the observed genotypic ratio from the expected genotypic ratio for zygotic SD test.

MapQTL v5.0 (Ooijen, 2011) was used to analysis of QTL data. Using an interval mapping approach; detection of QTLs was done by genetic linkage maps for each parent. Initially, 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. Putative QTLs with LOD scores greater than 2.0 in datasets were reported. The LOD thresholds at the genome-wide level were determined by running 1000 permutation test. In Siirt map genome-wide level were 2.9–3.6 for $P < 0.05$ and 3.7–4.6 for $P < 0.01$, while in Pa-18 map were 2.9–3.4 for $P < 0.05$ and 3.7–4.5 for $P < 0.01$. Furthermore, the Kruskal-Wallis test (KW test) was performed to verify the identified QTLs.

4. RESULTS AND DISSCUSSION

In this study, a genome survey study was used to estimate the genome size, GC content, and heterozygosity rates of pistachio (*P. vera* cv. Siirt). Genome-wide characterization of SSRs in the *P. vera* genome was also performed to develop novel SSR markers for pistachio. New SSRs were tested to determine transferability of SSR markers to other *Pistacia* species, and to reveal the population structure of *Pistacia* germplasm.

An inter-specific F1 populations of 'Siirt x Pa-18' were used to construct genetic linkage maps in pistachio. The parental maps as well as the consensus maps were constructed. Reference genetic linkage map was constructed with common markers as well. Moreover, previously identified sex-linked markers were used to determine positions in inter-specific F1 population. QTL analysis of leaf and shoot traits in pistachio were performed for the first time in inter-specific F1 populations.

4.1. Estimation Of Genome Size, Heterozygosity And GC Content

A total of 26.77 Gb clean data from 28.9 Gb raw sequences was used for K-mer analysis. A K-mer refers to an artificial sequence division of K nucleotides. A raw sequencing read with L bp contains (L-K+1) K-mers if the length of each K-mer is K bp. The frequency of each K-mer can be calculated from the raw genome sequencing reads. The K-mer frequencies along with the sequencing depth gradient follow a Poisson distribution in a given data set. During deduction, the genome size G= K_num/Peak_depth, where the K_num is the total number of K-mer and Peak_depth is the expected value of K-mer depth. Typically, K = 17. The 17-mer frequency distribution derived from the sequencing reads was plotted in Figure 4.1; the peak of the 17-mer distribution was about 28, and the total K-mer count was 16,684,162,450; therefore, the genome size of pistachio was estimated as 596 Mb. A small peak, observed at half the peak-depth, showed a high level of

heterozygosity for *P. vera*. Simulation of the *P. vera* genome with different heterozygosity rates showed it to be about 1% (Figure 4.1).

In the K-mer analysis the fat tail was not observed, therefore, the number of repeats in the pistachio genome may be low. The distribution of GC content versus sequencing depth (Figure 4.2) may provide information about sequencing bias. The GC content was about 37.1% in pistachio. There is also a region (red region) with an average depth around half that of main average depth, which may be caused by the high rate of heterozygosity.

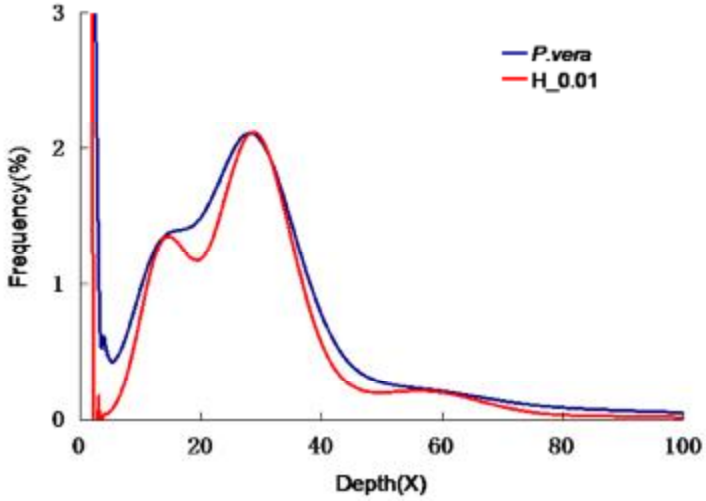


Figure 4.1. 17 K-mer analysis for estimating the genome size of *Pistacia vera* cv. Siirt. The X-axis is depth (X) and the Y-axis is the proportion that represents the frequency at that depth. The H_0.01 means that the heterozygous rate is 1%.

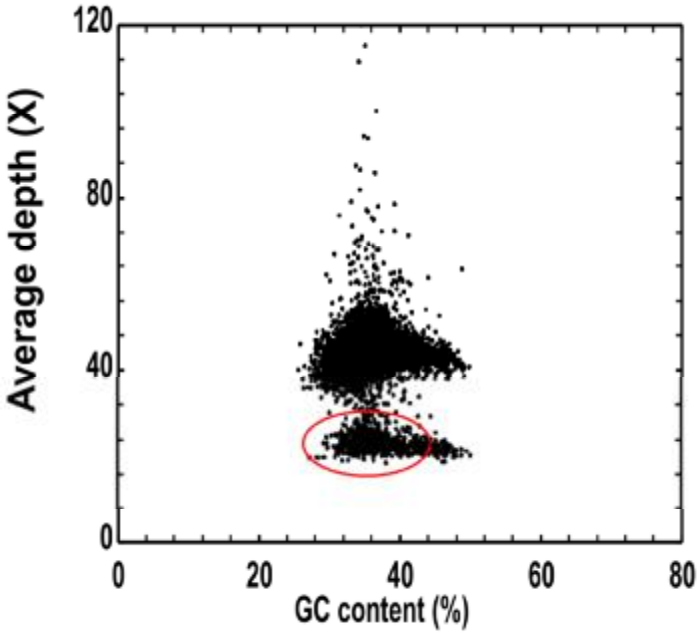


Figure 4.2. GC content and average sequencing depth. The X-axis represents GC content and the Y-axis represents the average depth. Red region whose average depth is around the half of main average depth, which may be caused by the high heterozygous rate.

The 17-nucleotide depth distribution suggested that the genome size of *P. vera* is about 600 Mb, which is close to the size (660 Mb) previously estimated for *P. terebinthus* using flow cytometry (Horjales et al, 2013). The estimated genome size of pistachio was found to be smaller than that of apple (Velasco et al, 2010), and larger than that of peach (Verde et al, 2013), sweet orange (Xu et al, 2013), and poplar (Tuskan et al, 2006). The small size of the pistachio genome may encourage scientists to perform whole genome sequencing in this species. The K-mer analysis also suggested a high level of heterozygosity for *P. vera*, which is probably due to the dioecious mating system in this genus.

The average GC content of the *P. vera* genome was higher than that of wild sweet potato (36.0%) (Hirakawa et al, 2015), but lower than that of switchgrass (45.5%) (Sharma et al, 2012) and Chinese jujube (48%) (Xiao et al, 2015).

Different GC contents may result in sequencing bias on the Illumina sequencing platform, and can, therefore, seriously affect genome assembly (Aird et al, 2011, Zhou et al 2013). GC content was one of the three factors found to contribute to sequencing bias on the Illumina sequencing platform (Cheung et al, 2011). High and low GC contents result in reduced coverage in sequencing regions (Zhou et al, 2013).

4.2. Development of Novel SSR Markers In *Pistacia*

4.2.1. Assembly And Identification Of SSR Loci In *P. vera*

Assembly was performed using 26.77 Gb Illumina PE (Pair-End) reads. The length of contig N_{50} was 2,327 bp, and the scaffold N_{50} was 3,399 bp long. The total length of scaffolds was 513.5 Mb. The number of scaffolds ≥100 bp was 893,901, and ≥2 kb was 44,900 (Table 4.1).

Table 4.1. The result of assembly in *P. vera* cv. Siirt using 26.77 Gb Illumina data

	Contigs		Scaffolds	
	Size(bp)	Number	Size(bp)	Number
N90	121	521,093	130	417,692
N80	262	230,751	442	163,608
N70	699	118,780	989	85,774
N60	1,325	67,518	1,874	47,596
N50[1]	2,327	39,274	3,399	27,069
Total Size	490,733,307	-	513,504,777	-
Total Number(≥=100bp)		980,753	-	893,901
Total Number(≥=2kb)		45,572	-	44,900

[1]The N_{50} of contigs or scaffolds was calculated by ordering all sequences, then adding the lengths from the longest to shortest until the added length exceeded 50% of the total length of all sequences. N_{60}, N_{70}, N_{80} and N_{90} were similarly defined.

A total of 59,280 di-, tri-, tetra-, penta-, and hexa-nucleotide SSR motifs (6, 5, 4, 4, and 4 repeat numbers, respectively) were detected, and the dinucleotide

motifs were the most abundant type of repeats (63.2%) in pistachio (Figure 4.3), followed by tri- (18.0%), tetra- (12.8%), penta- (3.8%), and hexanucleotide motifs (2.2%).

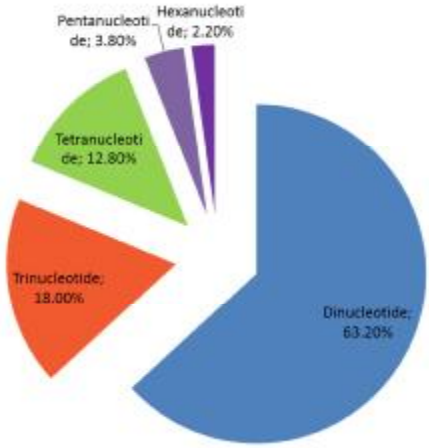


Figure 4.3. Distribution of 59,280 SSRs in the pistachio genome based on repeat type.

AT/AT (23.0%) and TA/TA (21.6%) were the most abundant repeats, and followed by AG/CT (7.6%) and GA/TC (6.2%), AAT/ATT (4.1%), CA/TG (2.6%), and TAA/ATT (2.6%) (Figure 4.4). The most abundant tetra- and penta-nucleotide repeat motif types were AAAT/ATTT (2.1%) and AAAAT/ATTTT (0.44%) respectively. Among hexanucleotide motifs, AAAAAT/ATTTTT and GCCCAA/TTGGGC (0.07%) were the most abundant motifs. The distribution of SSRs in the pistachio genome was calculated as one SSR per 8.67 kb.

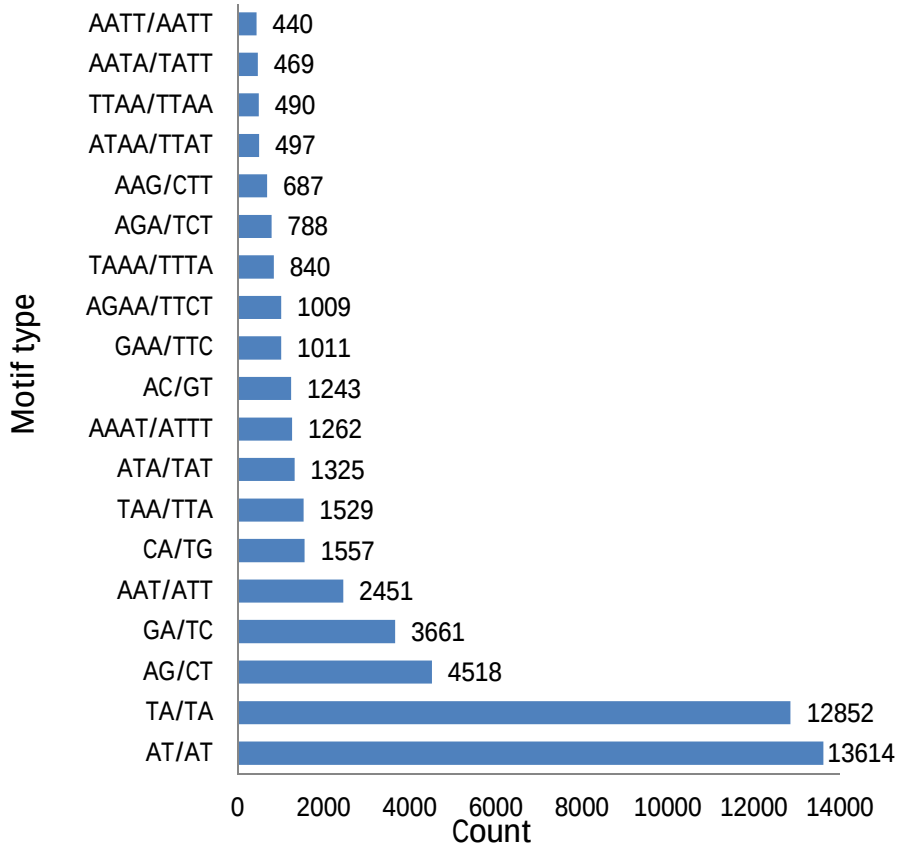


Figure 4.4. Distribution of SSR motifs in pistachio at 40x coverage sequencing data. The X-axis represents motif types and the Y-axis represents the count of motifs in the genome of pistachio.

4.2.2. Amplification And Polymorphism Level of SSRs

In the initial screening of 950 randomly selected SSR primer pairs using three (*Pistacia vera* L. cv. Siirt, *P. vera* cv. Bağyolu and one monoecious *Pistacia atlantica* Desf.) genotypes, 610 (64.2%) generated amplification products, 197 (20.7%) loci were monomorphic, and the remaining 143 (15.1%) SSR loci failed to generate amplification products. Of the amplified loci, 204 polymorphic and easily scorable SSR loci were selected to study genetic diversity in *Pistacia* (Figure 4. 5). Of them, 193 were perfect (94.6%), 8 (3.9%) were compound, and 3 (1.5%) were

interrupted repeats. Dinucleotide motifs were the most abundant (63.2%), followed by tri- (18.0%), hexa- (12.8%), tetra- (3.8%), and pentanucleotide motifs (2.2%). The sequences of 204 SSR loci were deposited into NCBI and were given in additional file 1 (GenBank accession numbers KX223398- KX223601; Table 4.2). Two SSR primer pairs (CUPVSiirt568 and CUPVSiirt689) amplified at two loci, and 206 SSR loci were obtained and used to study genetic diversity in *Pistacia*.

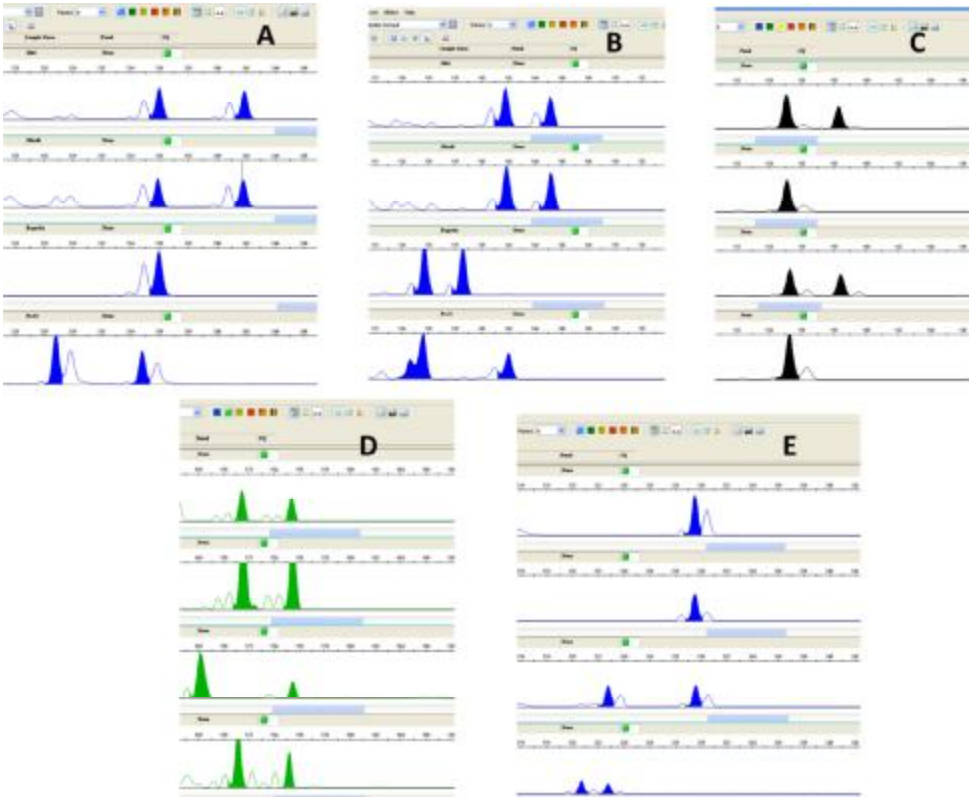


Figure 4.5. Electropherograms of SSR loci in Siirt, Ohadi , Bağyolu and Pa-18, respecively. A: CUPVSiirt446, B: CUPVSiirt543, C: CUPVSiirt724, D: CUPVSiirt1688 and E: CUPVSiirt1271.

Table 4.2. The forward and reverse primers, repeat motif, repeat type and product sizes of 204 novel SSR primer pairs developed from *Pistacia vera* L. cv Siirt.

No	SSR loci	Accession number	Sequence (5′–3′)	Sequence (5′–3′)	Repeat Motif	Repeat Type	Product size
1	CUPVSiirt15	KX223398	F: CAGAAAGCGCACTTAAAACCT	R: CCTCTCACTTCATAGTTGGGTGT	$(CT)_{13}$	Perfect	147
2	CUPVSiirt17	KX223399	F: TCCTTTATGAGGGAACACCTT	R: TGATGAATCAGCTTGTGCTACTG	$(TTA)_9$	Perfect	139
3	CUPVSiirt18	KX223400	F: AAGCCGGTCAAACTAGCTTAT	R: AAAATAGTACCTGCCCCCAAC	$(AAAT)_6$	Perfect	168
4	CUPVSiirt22	KX223401	F: CCGTGCCACAATTAATCCTAA	R: CGCGATGAATAGCAAGATTTC	$(AAG)_8$	Perfect	163
5	CUPVSiirt26	KX223402	F: CTGGGATTACTGATTTAGCAAAGG	R: TCCTACCTCCAACCTCCTAGC	$(ATG)_8$	Perfect	168
6	CUPVSiirt37	KX223403	F: TCAGGACAGTGGTGAAGGAGT	R: CTTTGGCTTCTCCAAGATTCA	$(TTA)_{10}$	Perfect	162
7	CUPVSiirt45	KX223404	F: CCAACTGTGAGTCCAAAACAGA	R: ATCGTCTGTTGCATTTGGCTA	$(AAGAG)_5$	Perfect	152
8	CUPVSiirt50	KX223405	F: TGCTAATGTCAGGTCACCAATC	R: CACAAGACAAAGAGATGCAGTCA	$(TA)_{13}$	Perfect	211
9	CUPVSiirt71	KX223406	F: GCAAAAGTAAATGTCCCAAAGG	R: TTCCCCTACAAACCATTAGCC	$(AAT)_8$	Perfect	143
10	CUPVSiirt76	KX223407	F: TTGCTCGAAGAGAGGTCAAAA	R: ATCCTCTTGGCATCCAAATTC	$(AT)_{15}$	Perfect	169
11	CUPVSiirt86	KX223408	F: CGAGCTGATTTATGGCTTAGAGA	R: TCCTCTCCTGGTTATGGGATT	$(AG)_{19}$	Perfect	131
12	CUPVSiirt95	KX223409	F: CACCCTCGTAACCAAATACCC	R: TCTGAGATTGTTGCCTGTATTC	$(ATA)_8$	Perfect	189
13	CUPVSiirt115	KX223410	F: GGTTCCGCTCTAGGTTTTAGG	R: CTGACCGATCGTGTGTTGATA	$(GGTTCC)_4$	Perfect	154
14	CUPVSiirt121	KX223411	F: TTTCATAGATCACAGTCGCTCA	R: ATTCAATTGGTTGGGGAGAGT	$(ATT)_7$	Perfect	169
15	CUPVSiirt125	KX223412	F: TGGCTGTCAAAACGTGAAAGT	R: CCAAAAGGTCATGGCAACTAA	$(TA)_{12}$	Perfect	159
16	CUPVSiirt129	KX223413	F: TCAAACCAAGGTGACAAGGAA	R: GGGAGAGGGATAGCAGTCTTG	$(AAT)_9$	Perfect	135
17	CUPVSiirt131	KX223414	F: CCAACCCTTCACCTTCTTGA	R: GGTATTGAGATGGCAGAGCAG	$(ACTCCG)_4$	Perfect	152
18	CUPVSiirt140	KX223415	F: GCAGCTTCAAATACAAGGATCA	R: ATGCATTTCCTCTTCATACGC	$(AGAGA)_5$	Perfect	246
19	CUPVSiirt149	KX223416	F: GTTTGGCTAATTATTGGAGGTG	R: GGCAGCTTCCATACACTTCTT	$(AT)_{12}$	Perfect	150
20	CUPVSiirt151	KX223417	F: CTTTTTCTTTGGCCCTCATCT	R: CCTTGTCTCTAATCCCTTGACC	$(TC)_{11}$	Perfect	141
21	CUPVSiirt158	KX223418	F: CGCACACATAGAAGACGGATAA	R: TACCCTTGCAATCAAACATGG	$(AT)_{11}$	Perfect	212
22	CUPVSiirt171	KX223419	F: TCTCGCCCTTAAAATAACACC	R: TAGTTTGGTAACAGCGGAGGA	$(TCT)_{11}$	Perfect	145

Table 4.2. Continued.

23	CUPVSiirt186	KX223420	F: CCTCTCTCCAGTTCATCTTCG	R: GATTTGAGTTGTGGTGGGACA	$(TTC)_{11}$	Perfect	147
24	CUPVSiirt213	KX223421	F: CGCATGCAAATGCTTTTAGTG	R: GCAAAGAGAAGGTGAGAGAAACA	$(CCTC)_6$	Perfect	196
25	CUPVSiirt230	KX223422	F: TGGGATGTTGCAGCTTCTTAT	R: CGCAACAAGCTTAACCCTTTA	$(AT)_{12}$	Perfect	169
26	CUPVSiirt238	KX223423	F: CGTGCAAGTTGGTCTGAATATC	R: CCTGCATTTCAGTAAACTCTTCG	$(AG)_{18}$	Perfect	178
27	CUPVSiirt242	KX223424	F: CAGCTTCATTACCCAGACCAC	R: TGTGAAACCGAACCCATATCT	$(AG)_{13}$	Perfect	143
28	CUPVSiirt243	KX223425	F: CATGGCAGCGTATGTGTTCTA	R: ATGTGGCAACCTAGAGGGAGT	$(TC)_{12}$	Perfect	130
29	CUPVSiirt256	KX223426	F: CGGCAAAATTTCATGGTACTC	R: AACTAGGAGGTCTGCGACTCTG	$(AG)_{12}$	Perfect	166
30	CUPVSiirt259	KX223427	F: CCTTCTTGTGGCCATACCATA	R: GCAAATAATGAATTCCCGCCTA	$(CT)_{14}(AT)_8$	Compound	251
31	CUPVSiirt265	KX223428	F: TAATTGCCCTTTTCACCTTG	R: GCATGGTTACTTACACCCCAAC	$(AAT)_8$	Perfect	180
32	CUPVSiirt271	KX223429	F: GATTCCCGCAGTTAAAGCAC	R: CACTTGGCCAGGGACTGTA	$(ACC)_9$	Perfect	158
33	CUPVSiirt284	KX223430	F: GCCAAAGACTGATGTGCTAAA	R: CTGGAAAAGTTAAGGTGAAGGAAC	$(GTAT)_6$	Perfect	219
34	CUPVSiirt294	KX223431	F: TCCTGCTGTGTTTGTGTTGAG	R: ATTTGTAGGTTCGTGCGTTTG	$(GA)_{11}$	Perfect	155
35	CUPVSiirt297	KX223432	F: TGGGCTGGTAGATTATCCGTA	R: TCCGTCCTTGTTATGTCTTTCC	$(AAAT)_6$	Perfect	157
36	CUPVSiirt298	KX223433	F: GCTAGCACCATAAACATTCAGC	R: ATACAATGTCATCGCCCCATA	$(AC)_{12}(AT)_8$	Compound	146
37	CUPVSiirt308	KX223434	F: AATAAACAGTCCACCCCAACC	R: TGGTGATTCTATCTCCTGCATT	$(GA)_{11}$	Perfect	158
38	CUPVSiirt312	KX223435	F: TATACCGGCCAGCTAAAGGTT	R: TTTCAGGCAGTCCAGGTCTATT	$(GATGAG)_4$	Perfect	149
39	CUPVSiirt316	KX223436	F: ATCAAACGGCATTGGAAATC	R: TGAGAATGCATGTGCAAACA	$(TA)_{11}$	Perfect	247
40	CUPVSiirt320	KX223437	F: ACTGCCCTTGAATCGAAAAAC	R: TTTACCACCACGAATTGGGTA	$(AAACAA)_4$	Perfect	163
41	CUPVSiirt328	KX223438	F: GTTTGAAAAGGGGTCAAAGGT	R: AGCGAATCCATTTGTGATGA	$(TTTC)_8$	Perfect	179
42	CUPVSiirt333	KX223439	F: GTGAGCAATGAATGTATGTG	R: GTGGATGTAGGAGGTTGTGGA	$(AG)_{11}$	Perfect	182
43	CUPVSiirt340	KX223440	F: CATGGATGAGGAAGCTCAGAC	R: AGACCTGTTGATCCCTGAATG	$(AT)_{12}$	Perfect	162
44	CUPVSiirt343	KX223441	F: TTTCTGTGTGGGTGTGTGAGA	R: TCCCTCTTCACACTTTCTTGC	$(TC)_{15}(AC)_6$	Perfect	159
45	CUPVSiirt349	KX223442	F: CAAACCCAGACATTGTGGACT	R: CATTGGTTGCATGCTTCTTCT	$(CAGACT)_4$	Perfect	148
46	CUPVSiirt357	KX223443	F: CAGTGCATATTCGGTGGACTT	R: AGTGAGGCTAGAATGCCCGTA	$(CACATC)_5$	Perfect	133
47	CUPVSiirt358	KX223444	F: GCGAGGAATAGGCACTATGAA	R: AAAAGGGAAGAGAAAGCAACG	$(TAT)_7$	Perfect	128

Table 4.2. Continued.

48	CUPVSiirt368	KX223445	F: CCACACAAAGAGAGGATCCAA	R: AACGTCTGTGAAAGAGAATCAG	$(TA)_{11}$	Perfect	138
49	CUPVSiirt415	KX223446	F: TGGTATTGTGCCAAGTCCTTT	R: CCACTATTGATGGACCAAAACC	$(TCAGGT)_5$	Perfect	151
50	CUPVSiirt436	KX223447	F: CCCGGTTAGCTCTCTCATGTA	R: AATGCTCTTCTTGTGGTCTCA	$(TTTTTC)_4$	Perfect	136
51	CUPVSiirt446	KX223448	F: TCTGGAACCCACAACAAGAAT	R: ATGTGGGATGGTTCACGTTTA	$(CTTGCA)_4$	Perfect	268
52	CUPVSiirt465	KX223449	F: CAACGTGCATTCCACAACTTA	R: GCTTAATTAGAGAGCTCGGGAAG	$(AT)_{12}$	Perfect	127
53	CUPVSiirt472	KX223450	F: GCAAATGCACAATGTATAGG	R: CAATTGAGTCCGATCAGGAAA	$(TTTC)_4$	Perfect	165
54	CUPVSiirt476	KX223451	F: GATGATTGGGAAGGGGGTATT	R: TCACATCCCGTTCTTTTATCC	$(TTA)_{12}$	Perfect	150
55	CUPVSiirt479	KX223452	F: GCTGCTTTGACCCACAATCTA	R: TGTCGAGTAATCCAGGCTTCTAA	$(TA)_{11}$	Perfect	151
56	CUPVSiirt496	KX223453	F: CCACCAAAAACTTGCAACACT	R: GATGGTTAATTCGCTTGCTTG	$(AAAC)_4$	Perfect	195
57	CUPVSiirt501	KX223454	F: ATCCTGCTCTTGGGAGAGGTA	R: ACCTTTCCATCTTTTCTCACG	$(AAGGGT)_4$	Perfect	133
58	CUPVSiirt505	KX223455	F: GGGGAATGCGCTTAATTACAA	R: GGAACTAAAGGAAGGGGGAAA	$(TTTTGT)_4$	Perfect	154
59	CUPVSiirt509	KX223456	F: ATCGCATGGTGTCATTAGGTT	R: CAGCAATTCACCACCTTTCAT	$(GCTGTG)_4$	Perfect	151
60	CUPVSiirt543	KX223457	F: CTTTAGTGTTTGGGCCAGCTA	R: GAATTCGGCAGACATAGACGA	$(ATA)_{11}$	Perfect	159
61	CUPVSiirt565	KX223458	F: GCTTCTTGCTCCTTTTTGTCAT	R: CCTCACTCTCTCCCTCCAAAC	$(AT)_{12}$	Perfect	161
62	CUPVSiirt568	KX223459	F: CCAACGAAAATGTTGGCTTT	R: TTGAGAAACCAGACCCTGATG	$(AAT)_8$	Perfect	165
63	CUPVSiirt569	KX223460	F: AAACATCAAGGAGCCTAACCA	R: AAGTTGCCGAACAACAGCTTA	$(ATTTTT)_4$	Perfect	130
64	CUPVSiirt598	KX223461	F: CAGGTACAACCTCTTGTGAACG	R: TGTAAACACACGTTAAGAGCAC	$(AT)_{12}$	Perfect	164
65	CUPVSiirt600	KX223462	F: GGAGTAATCACAAACCGAAACTG	R: AGCTTACCTCGATGGAACTTA	$(AG)_{15}$	Perfect	226
66	CUPVSiirt616	KX223463	F: CATTGGTATTGGGGGTTTTG	R: AGAGACCTATGCAATCGACCA	$(AT)_{11}$	Perfect	152
67	CUPVSiirt617	KX223464	F: TGGACACAAATGCCATCCTAT	R: TTGTGTTTGGTTCGTGTGGTA	$(ATA)_8(ACA)_5$	Compound	268
68	CUPVSiirt621	KX223465	F: CAATCTCATCTCATTTGCAGGA	R: AGTTTTTAGCCTGTCCTTTGC	$(AG)_{17}$	Perfect	143
69	CUPVSiirt625	KX223466	F: AATTACCAGCTTAGGCCCATC	R: TGCAAGGTACGAGACTTTTGG	$(TC)_{11}$	Perfect	148
70	CUPVSiirt649	KX223467	F: TCATTGAGGTTGAGGAGGTTG	R: CTTCCCCTTCTCGAAAACAAA	$(GGGATG)_4$	Perfect	144
71	CUPVSiirt660	KX223468	F: GACTGCAATCCCAACTCCATA	R: CTTCCTCTGCCATCCTCAAC	$(AG)_{11}$	Perfect	152
72	CUPVSiirt661	KX223469	F: TGGATAAGGATTGACCTTGATG	R: GCCATACCCATAAGACAATTACG	$(TG)_{12}$	Perfect	235

Table 4.2. Continued.

73	CUPVSint674	KX223470	F: CCATCCAGTATACGCAAAAACA	R: GGCCGTTAAATTAGAGCTATGC	$(AC)_{13}$	Perfect	232
74	CUPVSint689	KX223471	F: CCGCCGTTAGCAGATTAAGAT	R: CCTAAGAAAGGGTGGTATTCA	$(GT)_{14}AC(AT)_5$	Intrrupted	181
75	CUPVSint690	KX223472	F: GGCCAATTAAAATCTCCCCTTA	R: ATCTCGGTATTCGAGCTTTGC	$(AGCTCA)_4$	Perfect	209
76	CUPVSint712	KX223473	F: CCTGCACAAGTCGAGGAAAT	R: TTCGAGCGAGCTGATTTACAG	$(TC)_{11}$	Perfect	171
77	CUPVSint715	KX223474	F: CGTCTGTTCATTTCCTAACTATTCG	R: ATCCTGGAGGAACCAAGATGT	$(CA)_{13}(TA)_4$	Compound	142
78	CUPVSint719	KX223475	F: TTCACCCAATTAACATGCTACG	R: TAGAGTCGAACAGCGAAAGGA	$(TA)_{13}$	Perfect	194
79	CUPVSint724	KX223476	F: GCCTCAGCTCAGTCTCTTTGA	R: TGGAAAGAAGCCCTGAAAAAC	$(AGA)_8$	Perfect	148
80	CUPVSint742	KX223477	F: AGAGCATGCATAAAAGATGG	R: TCTGCACCTGAATGTGTTTGA	$(AAAAT)_6$	Perfect	200
81	CUPVSint743	KX223478	F: TAAGGGGTGGCCAAGAATTAC	R: CGGTTTTTAGGGCTAGGAGGT	$(AAAATC)_5$	Perfect	216
82	CUPVSint764	KX223479	F: ATCATTCCAAGCGTGCTTATC	R: GAGAAGGTTGAATAAAGGGTGA	$(ATGGAA)_6$	Perfect	212
83	CUPVSint768	KX223480	F: CTCAAGGTAATCCCTCGCTTT	R: ACCATGAATTCTTCCTTCG	$(TC)_{21}$	Perfect	239
84	CUPVSint782	KX223481	F: AGAACCGACTGGTTTTGGTTT	R: AACCTTCTTCCCCAAATCTCA	$(TG)_{15}$	Perfect	156
85	CUPVSint788	KX223482	F: ACCGAAGAAAACCGAGAAGAA	R: TCAAATAGGGGTCAAGGGTTT	$(AG)_{17}$	Perfect	224
86	CUPVSint794	KX223483	F: CGGATGCTCCTAAAAGTCTTGA	R: TGCTTCATACCGGTTCTTGAT	$(AAAG)_6$	Perfect	210
87	CUPVSint796	KX223484	F: CAATCTGTCTGCGCAATATCA	R: TTCTCCTGTTTGAGAGCAACAA	$(GA)_{12}$	Perfect	147
88	CUPVSint803	KX223485	F: GTCGTGGAAAATAAGAGTGGAA	R: TGAGAATATGGACCCACCAAA	$(TA)_{12}$	Perfect	190
89	CUPVSint818	KX223486	F: GTTGTTTGTGCATTTGGGTTC	R: TGCTGGAATAAAACGAGTTGC	$(TA)_{12}$	Perfect	166
90	CUPVSint836	KX223487	F: CCCCTCATATACAAGCCACAA	R: TGGGATTCACCTCATTCTCAA	$(AAAC)_8$	Perfect	195
91	CUPVSint838	KX223488	F: GCATCTAGGTTTTGGGCTTTC	R: ATTGGGGACCCACCTCTTTA	$(CT)_{18}$	Perfect	149
92	CUPVSint841	KX223489	F: ACCTGAGCCAACATAACCAGA	R: ACTTTGCTCCGGAATTTTCAC	$(GA)_{21}$	Perfect	192
93	CUPVSint847	KX223490	F: CTTGGCTACATCTCCCCTCTT	R: ACGACCACAAGGAAAGTGGTT	$(TAT)_{11}$	Perfect	230
94	CUPVSint855	KX223491	F: AGGAAGGGACGTCGTAGAAGA	R: TCTGTTGGCAGACTCTAGGTGA	$(GA)_{21}$	Perfect	242
95	CUPVSint858	KX223492	F: CCTCTAGGTTTTGCAGCTTTTC	R: GCTCTAGGGTTTGCAAGATTGA	$(AG)_8$	Perfect	180
96	CUPVSint875	KX223493	F: GGTTTCAACAAGGGAGTCAGC	R: AGTTAGACACACGGGGCTTTT	$(TA)_{12}$	Perfect	169
97	CUPVSint876	KX223494	F: TACCCTGGTAATGCTCGATCT	R: CAGGAACCAGCAGAAACAAAA	$(TTA)_{12}$	Perfect	186

Table 4.2. Continued.

98	CUPVSiirt883	KX223495	F: CAGGATTCAACCTCTCTAATCTG	R: GTCACACCCCACATAATGGAC	$(AT)_9(AG)_{17}$	Compound	167
99	CUPVSiirt889	KX223496	F: CTCATTGCCATGCATTACTGTT	R: TTGCATAGGACTACGAGAAAGC	$(AAT)_9$	Perfect	167
100	CUPVSiirt891	KX223497	F: ACGGAACCAGGAGGATCAGTA	R: TAATTGGGTCACGTCCTGTCT	$(CCCACA)_5$	Perfect	147
101	CUPVSiirt907	KX223498	F: ACACCATCTTCCAAACACTCC	R: GTAAGTGGGGGAAGAAGACGA	$(CAT)_9$	Perfect	155
102	CUPVSiirt924	KX223499	F: TTAGAAAAGAGACCGCCCATC	R: CTTGTATTCCATCCCCACCTT	$(TA)_{15}$	Perfect	159
103	CUPVSiirt929	KX223500	F: TAGACCCAAACACCCAGAAAA	R: TTAGGAGCAAGTTGGGCTTGT	$(AAAT)_7$	Perfect	134
104	CUPVSiirt931	KX223501	F: GGGATTTATTTGCCCAAGTTC	R: CCTCGACCCCTCTCATATTCT	$(AG)_{13}$	Perfect	135
105	CUPVSiirt932	KX223502	F: CACGAGGTTTTGCCTAGTGTC	R: GGCTAACTCATTAGGTGGAGGA	$(AG)_{13}TTAAAA(AG)_{12}$	Intrrupted	147
106	CUPVSiirt949	KX223503	F: GTTTCTTTGGCAGGAAAGAGC	R: CCAAATGCCCAATATGCTACA	$(AAT)_6$	Perfect	178
107	CUPVSiirt951	KX223504	F: CACATTCACAGCTTCGTTTCA	R: CATGCTGCAACAACACACTCT	$(GAA)_8$	Perfect	149
108	CUPVSiirt956	KX223505	F: CTCTGACAATGGGTGCCTTTA	R: GAGGCTTGTGGATATAGCTTCG	$(GA)_{15}$	Perfect	174
109	CUPVSiirt961	KX223506	F: GTGGAGAGACCAGGTGAAACA	R: CCCCTAACCCCTTTCTTTTCT	$(AG)_{13}$	Perfect	189
110	CUPVSiirt975	KX223507	F: AAGAGAGTGCTACTCGGGCTTA	R: GGGTGGGAACAAAACTATGCT	$(CTTT)_6$	Perfect	162
111	CUPVSiirt986	KX223508	F: TATCAACTGTGCGTTCCCATT	R: TAGACCAGCAGGACTGCCTAA	$(CATTCC)_5$	Perfect	150
112	CUPVSiirt989	KX223509	F: GCAACATGCAAACAAGTCCAT	R: CACAGAATCAATCTCCACTGTTC	$(AAAG)_6$	Perfect	163
113	CUPVSiirt1003	KX223510	F: ATCAATTGCTCCATCCATCAC	R: GATTTGGAAGCACAAAACTGC	$(TA)_{14}$	Perfect	142
114	CUPVSiirt1008	KX223511	F: CAGCATCAGCTTTTGATGTGA	R: CCACCCTTCTTCGACTTCTTA	$(GAT)_8$	Perfect	154
115	CUPVSiirt1017	KX223512	F: GGGGACCATCATTTGACTTTT	R: CCTTGGCTTCATGCGTTTAAT	$(GA)_{18}$	Perfect	219
116	CUPVSiirt1021	KX223513	F: AGCTCAAATACAACCCCGACT	R: AGCTGTGTCAAAATTGCTTCC	$(ATAA)_6$	Perfect	145
117	CUPVSiirt1041	KX223514	F: AACCCTAGGTGGGGCAAAT	R: CCACTGGACAGCTGGTACTCT	$(AG)_{11}$	Perfect	138
118	CUPVSiirt1043	KX223515	F: TCTTTCTCCATGTGTGCCTAAA	R: GTTCCTTCCCTCCAATTTGTC	$(ATT)_{10}$	Perfect	152
119	CUPVSiirt1047	KX223516	F: CCATTACTCCATGACCCTAATCA	R: GGGGATAGTTAAGCCTGTGGA	$(AAATA)_5$	Perfect	135
120	CUPVSiirt1053	KX223517	F: TGGTAGTTGCAAACATCTACATCAG	R: GCCAATTGTGTTGTGTAGAACC	$(GTGGCA)_4$	Perfect	147
121	CUPVSiirt1055	KX223518	F: AGGAGCAAGAGTGTTGTTTGC	R: CTTTACCTTTGGGAGGAGGTG	$(CTCCAC)_5$	Perfect	152
122	CUPVSiirt1057	KX223519	F: GTGACACACGAGGATGAGGTT	R: GCTTGTAGCCACCATTCAAAA	$(AC)_{11}$	Perfect	237

Table 4.2. Continued.

123	CUPVSsat1062	KX223520	F: TGAAAGGAAACCAGATCCTTG	R: TTTCCCATCACCTTCACTACG	$(AG)_{12}$	Perfect	125
124	CUPVSsat1071	KX223521	F: CATTAACGATTAGTCGGTGGTT	R: TGGTCTATTCATGCAAATACGG	$(AAT)_{10}$	Perfect	136
125	CUPVSsat1092	KX223522	F: GAACGGAATCTGAACCAAAGA	R: TCTTCTCGTATTTCCCAACCA	$(AG)_{12}$	Perfect	134
126	CUPVSsat1095	KX223523	F: GTGGGGAGATTTGAATTTTACG	R: TTTGGCCCTAACAATGATCT	$(TA)_{13}$	Perfect	226
127	CUPVSsat1116	KX223524	F: CGAGAACTCTGAATTGCAACA	R: GGAATCTTGTTGGAGGGGATA	$(AG)_{17}$	Perfect	156
128	CUPVSsat1117	KX223525	F: GCCACTTCACCATCAAACAAT	R: TCAACATCAGAGGTCAAAAG	$(AT)_{16}$	Perfect	177
129	CUPVSsat1120	KX223526	F: GTTTTGGTTGGTCCTGTGTTG	R: CTCTGCCTCAAGGTATGCACT	$(AT)_{12}$	Perfect	214
130	CUPVSsat1122	KX223527	F: CTTTGGGACGGACAATCATAA	R: GATTTAACAAAAGTCGGCCAAG	$(TA)_{11}$	Perfect	195
131	CUPVSsat1127	KX223528	F: GATGATCCCCCAAGTTGTATCG	R: TTGCAATGAGACCTTTTACCAG	$(TA)_{13}$	Perfect	143
132	CUPVSsat1140	KX223529	F: CATCAAATGCAACTGCAACTC	R: GCTACAATATCCCCTCCCTTTT	$(ATATA)_5$	Perfect	147
133	CUPVSsat1145	KX223530	F: CAGCAACTCCAAGGAATCAAA	R: TGTGTTGCGTTTAGCGAGTTA	$(CAA)_8$	Perfect	154
134	CUPVSsat1153	KX223531	F: CAGCGCTAACACTAAAAAAGATGG	R: CGCTGTCTCTCTCAACGGTAAA	$(CTCTTT)_4$	Perfect	177
135	CUPVSsat1163	KX223532	F: CATCCAGGTTGTTTCAGGTTC	R: TAGAATTAGTGCGCTGGGACA	$(TC)_{18}$	Perfect	146
136	CUPVSsat1171	KX223533	F: TGGGATAGTAGGTACCCAGTTG	R: CCCTGTGATCCATATTTTAACG	$(ATA)_8$	Perfect	236
137	CUPVSsat1182	KX223534	F: GAAGAAGCTAGAAGCGTCAGG	R: GGTGAAAGGGCTATGTTTCGT	$(CCTCAG)_4$	Perfect	157
138	CUPVSsat1183	KX223535	F: CAACCATTGGAGGAGCCTTT	R: GGTTGGGCTATACCGTCAAAT	$(AC)_{15}$	Perfect	273
139	CUPVSsat1188	KX223536	F: CTTGGTCCACCCAACACTATG	R: TTGGATTGCTTACTTGCTGGT	$(AAAAT)_5$	Perfect	153
140	CUPVSsat1189	KX223537	F: TTCTATAGCGTGGACAGCACA	R: TAGATCATCAGCGCCTAACCA	$(TA)_{11}$	Perfect	242
141	CUPVSsat1191	KX223538	F: CCGCAACACCAAATGGATA	R: ATCAAACGGAGCCTGAAGAAT	$(AT)_{15}$	Perfect	205
142	CUPVSsat1202	KX223539	F: CATTACTGAGCATGGATGCAAT	R: TGCTCTGTTTCACTCGATTTCT	$(AG)_{15}$	Perfect	168
143	CUPVSsat1214	KX223540	F: ACGACTGGCGCCTCTATAAAT	R: ACTCCCAGCGGTTATATTGCT	$(AG)_{11}$	Perfect	147
144	CUPVSsat1224	KX223541	F: TCGCCAACACCATCTTACTCT	R: AACAGCCTGAAGCCTTACTCA	$(GA)_{12}$	Perfect	247
145	CUPVSsat1238	KX223542	F: TACTGAGCCAATACACCATCC	R: TTTCATTCGTGTCATACGTAGC	$(ATA)_8$	Perfect	232
146	CUPVSsat1243	KX223543	F: CGGTCCTAAGAGAATGGGAGA	R: CCAGTTCTAGGAAGGGATTCA	$(TA)_{12}$	Perfect	132
147	CUPVSsat1250	KX223544	F: GATTTGAGGGTGTTTGGGTTA	R: CGCCCGGATGAATTACTTT	$(AT)_{11}$	Perfect	168

Table 4.2. Continued.

148	CUPVSint1260	KX223545	F: AAGCAGTCCCCAATTTAGAGC	R: ATCCTCGGCATGAACATATCA	$(AAT)_{12}$	Perfect	151
149	CUPVSint1267	KX223546	F: AAGAAGTTGTGGGCCTAGCAT	R: AGTCCCGATTTTTGACAGCTC	$(AAT)_9$	Perfect	122
150	CUPVSint1271	KX223547	F: TGAAACATGGAGAGGAAGCAT	R: CACCATTGCTTATTTCCCCTA	$(TAACCA)_4$	Perfect	214
151	CUPVSint1273	KX223548	F: TGATTGCTCGACATAGTTGGA	R: GATATCGGGGGTACTTGTGGT	$(ACCATT)_5$	Perfect	143
152	CUPVSint1278	KX223549	F: AGGACCACACCATTTCAATCA	R: TGCTTTCTTCACGCTCATTCT	$(ATA)_8$	Perfect	164
153	CUPVSint1322	KX223550	F: GCCCACTTCTTTTGATTTCCT	R: TAGCCTGTGAAGGAGGGAAGA	$(TC)_{12}$	Perfect	208
154	CUPVSint1326	KX223551	F: AGAGAGGGAGAAAACGCTTCA	R: GTCGCATGGAGATTGACTTTG	$(AG)_{16}$	Perfect	179
155	CUPVSint1330	KX223552	F: GATTTGCCAACTTTACGCTGA	R: CAAAGGTGAATCCTACGTCCA	$(CT)_{16}$	Perfect	152
156	CUPVSint1331	KX223553	F: CCCGAGCATGAAAATAATGG	R: CTTCACTTTACCCTTGTTGA	$(AAAT)_6$	Perfect	130
157	CUPVSint1345	KX223554	F: AATTGTTAATGGACGGGCTTC	R: ATGGTCATGGAAGGGGGTAAT	$(CCCATG)_5$	Perfect	175
158	CUPVSint1353	KX223555	F: GGCTTGTTTGAACTACCCTACC	R: GGGAATTACCCTCAACCAAAC	$(TC)_{21}$	Perfect	209
159	CUPVSint1360	KX223556	F: ATTTTGGTGATGTGGTTGCTC	R: GGCCTCTCAAACAAGCTCTAAA	$(CT)_{11}$	Perfect	156
160	CUPVSint1372	KX223557	F: CTATTGGGCCTTCCTCCACT	R: CTCGACCACTCAGAAGTAGTTAAAA	$(AT)_{11}$	Perfect	202
161	CUPVSint1378	KX223558	F: AAAGTCGCCGTTAGGACATCT	R: GTCGACAGTTTGAGGAGGTTG	$(TC)_{12}$	Perfect	149
162	CUPVSint1388	KX223559	F: CCGCTAGGAGCTCTGTGATTA	R: CGTCGTCGTTTTAACTCTTGG	$(GCGTTG)_4$	Perfect	178
163	CUPVSint1394	KX223560	F: AAGAAAAAGAGGCGAATCCTTG	R: ACAGTCAACATCTTCCGCAAT	$(AC)_7$	Perfect	256
164	CUPVSint1399	KX223561	F: TTGACTGCTGAAAAAGGAAGC	R: TCGATTTCTTCGCACACTTCT	$(AGCAGG)_3$	Perfect	174
165	CUPVSint1400	KX223562	F: TGGTCTGCTCCTGATGACTATG	R: GGAGGTAGCAACCCCTAAATC	$(TC)_{11}(AC)_{10}$	Compound	149
166	CUPVSint1402	KX223563	F: CTGGCCCATGATAATCTCCTT	R: TGGAAGCAGAAGACGAAAAGT	$(ATC)_8$	Perfect	169
167	CUPVSint1405	KX223564	F: CTTGCGTGATGAAGCACAAT	R: AGTTCCTCAAAGGGTTGATGT	$(AT)_{12}$	Perfect	208
168	CUPVSint1406	KX223565	F: ATCCTTCTGGCTTCCAATTTC	R: TGGATCTGTTTGCTGGACTCT	$(AATA)_6$	Perfect	177
169	CUPVSint1413	KX223566	F: GGTTGAAAGCTCCTCTTCCAT	R: CAACGAGTACAAGGAGAAGCAA	$(TTC)_8$	Perfect	156
170	CUPVSint1417	KX223567	F: GATGGGGATGCTAAATGACAA	R: GGGAATTAGGGTTGGGTCATA	$(AT)_{11}$	Perfect	163
171	CUPVSint1418	KX223568	F: GGCCCACGATTTTCTTTTTA	R: GAAGCGGTTACACTGTCCATT	$(AT)_{14}$	Perfect	135
172	CUPVSint1431	KX223569	F: TGGTTGCAAGGATGGATTAAC	R: TCACCCACTTTGAGAACATGA	$(CT)_{11}$	Perfect	196

Table 4.2. Continued.

173	CUPVSiirt1438	KX223570	F: TCAGCGAGTGGATAAGATT	R: TGTGTGGAATACCCACCAAGT	$(TAT)_{10}$	Perfect	256
174	CUPVSiirt1442	KX223571	F: TTTCTCACTCCGCTTTTCTCTT	R: CTCAGATGCGTTACAAACTAGGAA	$(AAAAT)_5$	Perfect	143
175	CUPVSiirt1457	KX223572	F: ATTCCACACTCGCAAATTCAG	R: GGTTTGGCTACAGATTGTACTGC	$(AC)_{13}(AT)_9$	Compound	163
176	CUPVSiirt1477	KX223573	F: CTCCATGAACGTCCTCCATTA	R: CCCAGGTGGCATATCCTCTAT	$(TCA)_8$	Perfect	157
177	CUPVSiirt1478	KX223574	F: TTCTATGGGTATCGGATTGGA	R: TATCGGATTGGATCACACACA	$(AC)_{13}(AT)_9$	Compound	137
178	CUPVSiirt1517	KX223575	F: TCTTCTTTCAAGGTGGGATTG	R: TGACTTTTAGCCCCCATATTCA	$(GAA)_8$	Perfect	247
179	CUPVSiirt1547	KX223576	F: TTCCGGCAATCACATATTCTC	R: CAAGAAATCGGCACTTAAGGA	$(TCCTTG)_4$	Perfect	144
180	CUPVSiirt1564	KX223577	F: CGCCAAACATTATCACAGGAC	R: GAAGCTAGAAAACAATGGAGGT	$(ATT)_8$	Perfect	250
181	CUPVSiirt1567	KX223578	F: GATGCGAAGATGCTATGGTTC	R: CACAATGCTTGCACGAGAGT	$(AT)_{12}$	Perfect	184
182	CUPVSiirt1599	KX223579	F: TCCTTAAGACGGGACCTAGAAA	R: CCTGTCCAAGAGTTAGGATTGTG	$(TA)_{11}$	Perfect	292
183	CUPVSiirt1611	KX223580	F: ATTAAATTGGCAGTGGCAGAG	R: GGGTTTTCAATGGGTTTTGA	$(TGGGT)_5$	Perfect	215
184	CUPVSiirt1626	KX223581	F: GGTCCCCTCAACACAACAAGT	R: TCTTTCTGCCTTGGAAACTCA	$(TC)_{13}$	Perfect	122
185	CUPVSiirt1628	KX223582	F: GCTTGCCAGCTGCCTATTAC	R: GGTTCATTGGAGAAATGATGG	$(CA)_{12}$	Perfect	164
186	CUPVSiirt1639	KX223583	F: ATATGACGTTTGGACCCCTTT	R: TTTGAACATGCCTGCCACT	$(AGTGGG)_4$	Perfect	170
187	CUPVSiirt1640	KX223584	F: ACCAATAAATCCAAGCCAAGC	R: CATGAAATGGTGCTTTTGAG	$(AC)_{13}$	Perfect	146
188	CUPVSiirt1652	KX223585	F: TCTCAGTTACAAACAGGCATCA	R: AAACAAGCTGAGAAGGAGCAG	$(AC)_{11}ATC(TA)_4$	Intrrupted	159
189	CUPVSiirt1655	KX223586	F: CATCATCATCATACCACAACCA	R: CTCTTCTTGACATTGGGAAAAC	$(TA)_{12}$	Perfect	139
190	CUPVSiirt1658	KX223587	F: GTGAGTCGTATGGGTGTTGGT	R: GATTACCTTGAACCAGCCAAA	$(GTGTGG)_4$	Perfect	144
191	CUPVSiirt1667	KX223588	F: GCCATTTGTATTGAGTTTGCAC	R: CCTCACTTGGGGACTACTTACC	$(CT)_{15}$	Perfect	150
192	CUPVSiirt1688	KX223589	F: GATTTCCGTGTCTATCCCACTT	R: GAGGATGTCATTCGGCTAAAA	$(AG)_{11}$	Perfect	154
193	CUPVSiirt1705	KX223590	F: TTATGCCGAGTATGAGTATC	R: CGGCCATATATACAGGCTCATT	$(GAGAT)_5$	Perfect	234
194	CUPVSiirt1714	KX223591	F: GCTCACTCGAAATGAAATCGT	R: CGAAGTCACAGATGGGAAAAA	$(TCATCT)_5$	Perfect	192
195	CUPVSiirt1734	KX223592	F: CTTAAGAATGAAGGCCCAAA	R: TGTACGGACAAGAAGGTGACG	$(AAT)_8$	Perfect	212
196	CUPVSiirt1740	KX223593	F: ACAAGGCCAAATAGAGCCAGT	R: ATATTCCAGCGCCCAGTAAGT	$(TCACTG)_5$	Perfect	157
197	CUPVSiirt1742	KX223594	F: GTGACAAGCTTCATTTTCATCG	R: CGATTTCCTCGCTTAATGTTC	$(AT)_{17}$	Perfect	193

Table 4.2. Continued.

198	CUPVSint1749	KX223595	F: CAAGACACAGTTTTCTGCCAAT	R: CTGTTTTTGGGCTTCTTTCG	$(GAAACG)_5$	Perfect	149
199	CUPVSint1759	KX223596	F: GCTCGAAATCTAACACCACCTT	R: AGATTCTCTGGTTGGGTTTGG	$(CT)_{12}$	Perfect	159
200	CUPVSint1764	KX223597	F: TTCCTCTGCTAACACCTGCAC	R: TGATTCCGGCTGTAGATTTCTT	$(AAT)_{10}$	Perfect	151
201	CUPVSint1768	KX223598	F: AAGGGGTCAAACTCAAAGTGAA	R: ATGATATCCCTTTCCCCCTTT	$(GA)_{13}$	Perfect	150
202	CUPVSint1784	KX223599	F: TCAAGATCCTCATATTCCTAGCC	R: CCATTATCAGATGCCCTGTGT	$(TAA)_9$	Perfect	222
203	CUPVSint1788	KX223600	F: AGCAGAAGCAAGAAAGGGTTC	R: TTCCAGAACTCAAGGCTATT	$(GA)_{11}$	Perfect	170
204	CUPVSint1797	KX223601	F: CTCTCTCCGGCCACTAATTCT	R: TGAACTGGTTTGACTGCTCTTC	$(CT)_{13}$	Perfect	149

4.2.3. Diversity Measures Of Novel SSR Loci In *Pistacia*

Genetic diversity was studied by analyzing 24 pistachio cultivars and 20 wild *Pistacia* genotypes (four genotypes from each five wild *Pistacia* species) belonging to *P. atlantica*, *Pistacia integerrima* Stewart., *Pistacia chinenesis* Bunge, *Pistacia terebinthus* L., and *Pistacia lentiscus* L. genotypes. Allele ranges, number of alleles (*Na*), effective number of alleles (*Ne*), polymorphism information content (*PIC*), expected (*He*), and observed (*Ho*) heterozygosities of the 206 SSR loci are presented in Table 4.3.

Table 4.3. Novel SSR loci with genetic diversity measures in 44 *Pistacia* genotypes: allele ranges, number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and PIC values of 206 loci

No	SSR Loci	*Na*	*Ne*	*Ho*	*He*	*PIC*	Allele ranges (bp)
1	CUPVSiirt15[a]	10	5.12	0.45	0.80	0.79	96-123
2	CUPVSiirt17	2	1.26	0.00	0.20	0.18	103-109
3	CUPVSiirt18	2	1.09	0.09	0.08	0.08	181-185
4	CUPVSiirt22a	13	8.64	0.46	0.88	0.87	134-182
5	CUPVSiirt26[ab]	9	4.39	0.39	0.77	0.74	164-190
6	CUPVSiirt37[ab]	14	7.43	0.60	0.87	0.85	140-167
7	CUPVSiirt45[a]	2	1.89	0.12	0.47	0.36	123-133
8	CUPVSiirt50	10	4.94	0.33	0.80	0.77	155-190
9	CUPVSiirt71[a]	11	5.75	0.37	0.83	0.81	128-168
10	CUPVSiirt76[a]	8	5.72	0.29	0.83	0.80	153-188
11	CUPVSiirt86[a]	19	8.96	0.82	0.89	0.88	111-159
12	CUPVSiirt95[a]	13	5.74	0.40	0.83	0.81	191-235
13	CUPVSiirt115	6	5.94	0.28	0.83	0.81	166-173
14	CUPVSiirt121	6	2.45	0.10	0.59	0.55	106-143
15	CUPVSiirt125[a]	12	6.73	0.57	0.85	0.83	167-224

Table 4.3. Continued

16	CUPVSiirt129[ab]	16	6.76	0.59	0.85	0.84	127-172
17	CUPVSiirt131[a]	8	2.96	0.38	0.66	0.62	156-175
18	CUPVSiirt140[a]	7	5.10	0.33	0.80	0.78	213-235
19	CUPVSiirt149[a]	15	5.25	0.43	0.81	0.79	98-136
20	CUPVSiirt151	9	4.17	0.40	0.76	0.73	150-172
21	CUPVSiirt158[a]	9	6.27	0.47	0.84	0.82	121-243
22	CUPVSiirt171[a]	4	2.74	0.25	0.64	0.59	138-161
23	CUPVSiirt186[a]	15	4.42	0.56	0.77	0.76	140-182
24	CUPVSiirt213[a]	7	2.57	0.34	0.61	0.58	205-223
25	CUPVSiirt230[a]	12	4.77	0.30	0.79	0.77	177-216
26	CUPVSiirt238	8	3.80	0.37	0.74	0.70	171-195
27	CUPVSiirt242[a]	13	6.13	0.49	0.84	0.82	129-167
28	CUPVSiirt243[a]	13	4.80	0.41	0.79	0.77	137-166
29	CUPVSiirt256[ab]	7	3.20	0.36	0.69	0.63	171-199
30	CUPVSiirt259[a]	10	5.33	0.58	0.81	0.79	186-218
31	CUPVSiirt265[a]	5	2.97	0.17	0.66	0.62	111-200
32	CUPVSiirt271	10	5.24	0.67	0.81	0.78	96-118
33	CUPVSiirt284[ab]	12	6.08	0.39	0.84	0.82	220-256
34	CUPVSiirt294[a]	10	6.24	0.34	0.84	0.82	114-138
35	CUPVSiirt297[a]	8	5.51	0.30	0.82	0.79	136-146
36	CUPVSiirt298	18	6.86	0.67	0.85	0.84	106-198
37	CUPVSiirt308[a]	8	4.62	0.33	0.78	0.76	166-175
38	CUPVSiirt312[a]	8	2.27	0.29	0.56	0.54	154-172
39	CUPVSiirt316	7	2.87	0.31	0.65	0.62	254-280
40	CUPVSiirt320[a]	2	1.83	0.50	0.45	0.35	173-179
41	CUPVSiirt328	6	2.52	0.40	0.60	0.58	181-204
42	CUPVSiirt333[a]	11	5.92	0.51	0.83	0.81	127-159
43	CUPVSiirt340[a]	12	6.45	0.48	0.85	0.83	125-171
44	CUPVSiirt343	4	1.96	0.33	0.49	0.43	121-127
45	CUPVSiirt349[ab]	13	5.69	0.63	0.82	0.8	164-199

Table 4.3. Continued

46	CUPVSiirt357[a]	14	9.24	0.60	0.89	0.88	181-231
47	CUPVSiirt358	9	5.80	0.27	0.83	0.81	215-269
48	CUPVSiirt368	7	2.49	0.03	0.60	0.57	152-188
49	CUPVSiirt415	6	3.59	0.28	0.72	0.68	140-170
50	CUPVSiirt436[ab]	13	6.01	0.68	0.83	0.82	91-118
51	CUPVSiirt446[a]	4	1.96	0.36	0.49	0.42	221-242
52	CUPVSiirt465[a]	16	9.93	0.45	0.90	0.89	112-178
53	CUPVSiirt472[a]	16	4.67	0.44	0.79	0.77	172-325
54	CUPVSiirt476[ab]	9	6.01	0.43	0.83	0.82	145-171
55	CUPVSiirt479	5	2.01	0.48	0.50	0.46	165-192
56	CUPVSiirt496	14	3.94	0.26	0.75	0.73	158-239
57	CUPVSiirt501	6	2.26	0.34	0.56	0.52	146-171
58	CUPVSiirt505[ab]	13	4.20	0.43	0.76	0.74	146-186
59	CUPVSiirt509[ab]	12	3.28	0.36	0.69	0.67	162-188
60	CUPVSiirt543[a]	11	6.76	0.63	0.85	0.84	111-149
61	CUPVSiirt565[ab]	16	7.49	0.43	0.87	0.85	134-174
62	CUPVSiirt568x	5	2.46	0.21	0.59	0.52	100-117
63	CUPVSiirt568y	15	8.50	0.48	0.88	0.87	117-145
64	CUPVSiirt569[a]	5	3.99	0.62	0.75	0.71	95-109
65	CUPVSiirt598	14	7.38	0.60	0.86	0.85	163-204
66	CUPVSiirt600	8	5.17	0.54	0.81	0.78	225-241
67	CUPVSiirt616	13	9.88	0.51	0.90	0.89	98-204
68	CUPVSiirt617	4	3.12	0.39	0.68	0.62	277-290
69	CUPVSiirt621[a]	9	3.98	0.33	0.75	0.72	93-129
70	CUPVSiirt625[a]	15	9.05	0.36	0.89	0.88	152-191
71	CUPVSiirt649[a]	4	2.68	0.47	0.63	0.56	158-177
72	CUPVSiirt660[ab]	10	3.89	0.62	0.74	0.71	129-146
73	CUPVSiirt661	7	3.28	0.44	0.70	0.67	209-288
74	CUPVSiirt674	6	2.60	0.32	0.61	0.57	235-249
75	CUPVSiirt689x	5	2.10	0.38	0.52	0.50	200-210

Table 4.3. Continued

76	CUPVSiirt689y	4	2.18	0.49	0.54	0.45	306-314
77	CUPVSiirt690	2	1.40	0.16	0.28	0.24	215-221
78	CUPVSiirt712	9	3.07	0.46	0.67	0.64	180-210
79	CUPVSiirt715	9	2.64	0.25	0.62	0.59	139-176
80	CUPVSiirt719[ab]	13	6.99	0.71	0.86	0.84	190-234
81	CUPVSiirt724	3	2.88	0.38	0.65	0.58	132-138
82	CUPVSiirt742[a]	14	3.19	0.30	0.69	0.67	196-232
83	CUPVSiirt743	12	6.70	0.39	0.85	0.83	159-205
84	CUPVSiirt764[a]	9	6.14	0.56	0.84	0.82	161-206
85	CUPVSiirt768[ab]	6	3.43	0.60	0.71	0.67	206-220
86	CUPVSiirt782[ab]	10	4.41	0.5	0.77	0.75	172-202
87	CUPVSiirt788	16	6.48	0.43	0.85	0.83	205-262
88	CUPVSiirt794	14	5.90	0.65	0.83	0.81	206-244
89	CUPVSiirt796[ab]	17	8.32	0.66	0.88	0.87	92-130
90	CUPVSiirt803	8	3.49	0.36	0.71	0.68	202-266
91	CUPVSiirt818[a]	10	5.65	0.48	0.82	0.80	165-187
92	CUPVSiirt836[a]	13	2.69	0.23	0.63	0.61	144-174
93	CUPVSiirt838[ab]	14	6.21	0.76	0.84	0.82	139-181
94	CUPVSiirt841	14	5.81	0.59	0.83	0.81	146-186
95	CUPVSiirt847	9	6.13	0.50	0.84	0.82	226-256
96	CUPVSiirt855[a]	9	4.17	0.43	0.76	0.74	237-278
97	CUPVSiirt858[a]	11	4.62	0.63	0.78	0.76	167-198
98	CUPVSiirt875	13	4.23	0.28	0.76	0.74	157-190
99	CUPVSiirt876[ab]	16	8.05	0.77	0.88	0.86	180-221
100	CUPVSiirt883	12	3.69	0.28	0.73	0.71	172-219
101	CUPVSiirt889[a]	8	4.57	0.40	0.78	0.75	168-188
102	CUPVSiirt891[a]	6	3.82	0.28	0.74	0.70	130-168
103	CUPVSiirt907	3	2.86	0.45	0.65	0.58	92-98
104	CUPVSiirt924[a]	4	1.49	0.00	0.33	0.31	81-89
105	CUPVSiirt929[a]	13	5.66	0.40	0.82	0.81	81-105

Table 4.3. Continued

106	CUPVSiirt931[a]	7	4.59	0.12	0.78	0.75	146-340
107	CUPVSiirt932	7	3.48	0.43	0.71	0.67	123-132
108	CUPVSiirt949[a]	9	4.31	0.34	0.77	0.74	160-197
109	CUPVSiirt951	6	1.78	0.08	0.44	0.42	154-184
110	CUPVSiirt956[a]	18	6.31	0.42	0.84	0.83	105-200
111	CUPVSiirt961[a]	10	5.85	0.33	0.83	0.81	177-210
112	CUPVSiirt975[a]	6	3.44	0.30	0.71	0.66	139-154
113	CUPVSiirt986[a]	10	3.09	0.41	0.68	0.65	146-169
114	CUPVSiirt989[a]	13	4.10	0.32	0.76	0.74	140-176
115	CUPVSiirt1003[ab]	12	7.87	0.49	0.87	0.86	71-112
116	CUPVSiirt1008[a]	6	2.93	0.20	0.66	0.60	163-174
117	CUPVSiirt1017[a]	16	6.14	0.50	0.84	0.82	203-245
118	CUPVSiirt1021[a]	6	3.03	0.57	0.67	0.63	123-143
119	CUPVSiirt1041[a]	7	4.62	0.40	0.78	0.75	100-114
120	CUPVSiirt1043[a]	13	3.43	0.30	0.71	0.69	89-157
121	CUPVSiirt1047[ab]	11	3.74	0.34	0.73	0.72	125-157
122	CUPVSiirt1053[a]	9	4.05	0.40	0.75	0.72	145-189
123	CUPVSiirt1055[a]	7	2.83	0.24	0.65	0.62	151-180
124	CUPVSiirt1057[a]	12	5.05	0.42	0.80	0.78	226-258
125	CUPVSiirt1062[a]	12	3.84	0.56	0.74	0.71	138-172
126	CUPVSiirt1071[ab]	16	7.69	0.63	0.87	0.86	123-169
127	CUPVSiirt1092[a]	11	6.15	0.56	0.84	0.82	136-165
128	CUPVSiirt1095	11	7.78	0.58	0.87	0.86	126-290
129	CUPVSiirt1116[a]	15	5.37	0.57	0.81	0.80	151-191
130	CUPVSiirt1117[a]	12	7.74	0.56	0.87	0.86	143-172
131	CUPVSiirt1120[ab]	11	4.50	0.39	0.78	0.75	193-218
132	CUPVSiirt1122	7	5.54	0.45	0.82	0.80	121-215
133	CUPVSiirt1127	9	4.74	0.50	0.79	0.77	118-142
134	CUPVSiirt1140[a]	8	5.94	0.40	0.83	0.81	156-176
135	CUPVSiirt1145[ab]	12	5.98	0.58	0.83	0.81	150-174

Table 4.3. Continued

136	CUPVSiirt1153[a]	8	3.96	0.26	0.75	0.72	172-201
137	CUPVSiirt1163	7	3.80	0.43	0.74	0.70	140-166
138	CUPVSiirt1171	14	3.07	0.38	0.67	0.66	229-270
139	CUPVSiirt1182[ab]	8	4.46	0.43	0.78	0.75	155-179
140	CUPVSiirt1183	7	4.48	0.33	0.78	0.75	235-250
141	CUPVSiirt1188	3	2.56	0.31	0.61	0.54	116-168
142	CUPVSiirt1189	5	2.56	0.36	0.61	0.56	247-267
143	CUPVSiirt1191	5	3.19	0.46	0.69	0.65	154-190
144	CUPVSiirt1202[ab]	16	4.18	0.50	0.76	0.74	174-212
145	CUPVSiirt1214[a]	10	4.56	0.15	0.78	0.76	157-182
146	CUPVSiirt1224[ab]	9	6.37	0.53	0.84	0.82	257-292
147	CUPVSiirt1238[ab]	11	3.88	0.45	0.74	0.72	229-258
148	CUPVSiirt1243[ab]	14	6.64	0.20	0.85	0.84	134-164
149	CUPVSiirt1250[a]	10	4.77	0.40	0.79	0.76	175-192
150	CUPVSiirt1260[a]	10	3.21	0.36	0.69	0.66	151-184
151	CUPVSiirt1267	9	4.78	0.49	0.79	0.77	124-155
152	CUPVSiirt1271[a]	10	3.76	0.23	0.73	0.72	203-234
153	CUPVSiirt1273[a]	6	2.53	0.75	0.61	0.55	135-160
154	CUPVSiirt1278[a]	11	2.90	0.21	0.66	0.63	176-220
155	CUPVSiirt1322[a]	9	3.83	0.40	0.74	0.71	218-237
156	CUPVSiirt1326[ab]	17	7.26	0.55	0.86	0.85	186-212
157	CUPVSiirt1330	18	10.58	0.71	0.91	0.90	154-201
158	CUPVSiirt1331	11	4.23	0.34	0.76	0.74	97-119
159	CUPVSiirt1345	7	2.46	0.38	0.59	0.55	156-191
160	CUPVSiirt1353	10	4.70	0.53	0.79	0.76	169-197
161	CUPVSiirt1360	5	3.59	0.34	0.72	0.67	127-135
162	CUPVSiirt1372[ab]	11	4.53	0.20	0.78	0.76	112-194
163	CUPVSiirt1378	11	4.06	0.30	0.75	0.72	83-119
164	CUPVSiirt1388[a]	6	4.67	0.43	0.79	0.75	177-201
165	CUPVSiirt1394[a]	9	5.17	0.28	0.81	0.78	228-270

Table 4.3. Continued

166	CUPVSiirt1399	6	1.95	0.18	0.49	0.46	183-216
167	CUPVSiirt1400[ab]	17	5.34	0.77	0.81	0.80	155-197
168	CUPVSiirt1402[ab]	9	4.36	0.45	0.77	0.74	172-213
169	CUPVSiirt1405[ab]	18	8.85	0.56	0.89	0.88	176-223
170	CUPVSiirt1406	13	3.81	0.23	0.74	0.72	172-208
171	CUPVSiirt1413[a]	10	3.74	0.46	0.73	0.70	166-202
172	CUPVSiirt1417	12	4.84	0.31	0.79	0.78	134-374
173	CUPVSiirt1418[a]	16	8.24	0.37	0.88	0.87	114-159
174	CUPVSiirt1431[a]	8	3.65	0.29	0.73	0.70	198-215
175	CUPVSiirt1438[a]	9	4.06	0.14	0.75	0.72	265-290
176	CUPVSiirt1442[a]	12	4.12	0.27	0.76	0.74	110-138
177	CUPVSiirt1457[a]	12	7.84	0.32	0.87	0.86	149-199
178	CUPVSiirt1477[ab]	12	6.27	0.57	0.84	0.83	109-126
179	CUPVSiirt1478	11	4.77	0.46	0.79	0.76	84-119
180	CUPVSiirt1517[a]	10	5.13	0.21	0.8	0.79	213-225
181	CUPVSiirt1547[a]	2	1.38	0.18	0.28	0.24	120-126
182	CUPVSiirt1564	12	5.94	0.34	0.83	0.81	188-222
183	CUPVSiirt1567[ab]	15	5.69	0.52	0.82	0.81	181-215
184	CUPVSiirt1599	11	6.53	0.39	0.85	0.83	292-345
185	CUPVSiirt1611[ab]	8	2.68	0.16	0.63	0.6	193-215
186	CUPVSiirt1626[a]	12	7.14	0.27	0.86	0.85	114-153
187	CUPVSiirt1628	7	2.98	0.40	0.66	0.61	125-155
188	CUPVSiirt1639[a]	7	4.60	0.39	0.78	0.75	167-194
189	CUPVSiirt1640[a]	10	3.45	0.17	0.71	0.67	134-180
190	CUPVSiirt1652[ab]	13	5.30	0.61	0.81	0.79	159-193
191	CUPVSiirt1655[ab]	14	5.77	0.53	0.83	0.81	150-213
192	CUPVSiirt1658[a]	13	3.89	0.44	0.74	0.72	130-209
193	CUPVSiirt1667[ab]	12	4.58	0.42	0.78	0.76	149-186
194	CUPVSiirt1688	11	5.74	0.63	0.83	0.80	160-184
195	CUPVSiirt1705	3	1.35	0.06	0.26	0.24	231-251

Table 4.3. Continued

196	CUPVSiirt1714	5	2.13	0.08	0.53	0.50	196-208
197	CUPVSiirt1734	5	3.24	0.50	0.69	0.65	168-185
198	CUPVSiirt1740[a]	7	2.88	0.41	0.65	0.61	157-192
199	CUPVSiirt1742[a]	15	7.32	0.25	0.86	0.85	149-213
200	CUPVSiirt1749[ab]	9	4.44	0.52	0.77	0.74	133-164
201	CUPVSiirt1759[ab]	11	5.79	0.62	0.83	0.81	137-159
202	CUPVSiirt1764[a]	13	3.59	0.43	0.72	0.71	154-178
203	CUPVSiirt1768[a]	8	3.63	0.45	0.72	0.69	108-128
204	CUPVSiirt1784[a]	9	5.46	0.43	0.82	0.79	177-211
205	CUPVSiirt1788[a]	4	2.44	0.14	0.59	0.51	172-189
206	CUPVSiirt1797[a]	13	4.98	0.45	0.80	0.78	143-177
	Total	**2,036**	**-**	**-**	**-**	**-**	
	Mean	**9.88**	**4.67**	**0.41**	**0.74**	**0.71**	**71-374**

[a]had amplifications in six *Pistacia* species
[b]were polymorphic in six *Pistacia* species

A total of 2,036 alleles were produced by 206 SSR loci in the characterization of 44 *Pistacia* cultivars and genotypes, ranging from 2 to 19 per locus. The highest number of alleles was obtained from the CUPVSiirt86 locus. The CUPVSiirt298, CUPVSiirt956, CUPVSiirt1330, and CUPVSiirt1405 loci also produced a high number of alleles (*Na* = 18). The effective number of alleles ranged from 1.09 (CUPVSiirt18) to 10.58 (CUPVSiirt1330) with an average of Ne = 4.67. The CUPVSiirt465 (*Ne* = 9.93), CUPVSiirt616 (*Ne* = 9.88), CUPVSiirt357 (*Ne* = 9.24), and CUPVSiirt625 (*Ne* = 9.05) loci also had high effective numbers of alleles. The observed heterozygosity (*Ho*) ranged from 0.0 to 0.82 with an average of *Ho* = 0.41. The CUPVSiirt86 locus was the most heterozygous, whereas the CUPVSiirt17 and CUPVSiirt924 loci were homozygous. The average *He* value was 0.74, and ranged between 0.08 (CUPVSiirt18) and 0.91 (CUPVSiirt1330). The *PIC* values ranged from 0.08 to 0.90 with an average of 0.71 (Table 4.3).

4.2.4. Diversity Of The SSRs In Each Of Six *Pistacia* Species

In *P. vera*, all 206 SSR loci generated amplification products, and a total of 897 alleles were produced with an average of 4.5 alleles per locus. Two hundred (97.1%) SSR loci were polymorphic in 24 pistachio cultivars. Number of allele (*Na*), effective number of alleles (*Ne*), observed heterozigosity (*Ho*), expected heterozygosity (*He*), polymorphic information content (*PIC*) value and allele range are calculated for each locus. The number of allele ranged between two and eleven and the highest number of allele (*Na* = 11) was obtained from the CUPVSiirt1330 locus. The CUPVSiirt568y, CUPVSiirt956 and CUPVSiirt1418 loci also produced a high number of alleles (*Na* = 10). The effective number of alleles ranged from 1.04 (CUPVSiirt121 and CUPVSiirt1547) to 7.60 (CUPVSiirt616) with an average of 2.57. The high effective numbers of alleles were also obtained in CUPVSiirt357 (*Ne* = 5.60), CUPVSiirt358 (*Ne* = 5.80), CUPVSiirt1330 (*Ne* = 6.26), and CUPVSiirt625 (Ne = 9.05) loci. The observed heterozigosity (*Ho*) value ranged from 0.00 to 1.00. The average observed heterozygosity (*Ho*) was 0.46, and the CUPVSiirt86 and CUPVSiirt1273 loci were the most heterozygous, whereas CUPVSiirt1047 gave homozygous patterns in the amplification, and having Ho = 0.00. The expected heterozygosity (*He*) values varied from 0.04 to 0.87 with an average of 0.55. The CUPVSiirt616 locus was more informative than the others due to their higher expected heterozygosity values, while CUPVSiirt121 and CUPVSiirt1547 provided less informative value. The polymorphisim information content (*PIC*) values ranged between 0.04 (CUPVSiirt121 and CUPVSiirt1547) and 0.85 (CUPVSiirt616) with an average of 0.50 (Table 4.4).

Table 4.4. Number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), PIC values and allele range of 200 polymorphic SSR loci in *P. vera*.

No	Loci	*Na*	*Ne*	*Ho*	*He*	*PIC*	Allele range (bp)
1	CUPVSiirt15	4	2.00	0.63	0.50	0.45	106-115
2	CUPVSiirt18	2	1.09	0.09	0.08	0.08	181-185
3	CUPVSiirt22	8	3.54	0.53	0.72	0.69	162-180
4	CUPVSiirt26	5	3.01	0.30	0.67	0.60	179-190
5	CUPVSiirt37	6	2.98	0.63	0.66	0.61	143-157
6	CUPVSiirt45	2	1.63	0.24	0.39	0.31	123-133
7	CUPVSiirt50	6	3.29	0.41	0.70	0.66	162-190
8	CUPVSiirt71	6	2.41	0.68	0.59	0.56	156-168
9	CUPVSiirt76	4	2.78	0.27	0.64	0.59	153-188
10	CUPVSiirt86	9	5.10	1.00	0.80	0.78	142-159
11	CUPVSiirt95	6	3.35	0.46	0.70	0.67	193-226
12	CUPVSiirt115	5	4.66	0.25	0.79	0.75	169-173
13	CUPVSiirt121	2	1.04	0.04	0.04	0.04	106-143
14	CUPVSiirt125	5	3.65	0.75	0.73	0.68	167-187
15	CUPVSiirt129	4	2.84	0.46	0.65	0.58	141-163
16	CUPVSiirt131	2	1.44	0.38	0.30	0.26	163-169
17	CUPVSiirt140	4	2.92	0.50	0.66	0.59	215-235
18	CUPVSiirt149	4	1.98	0.38	0.50	0.42	105-116
19	CUPVSiirt151	3	2.17	0.42	0.54	0.47	154-166
20	CUPVSiirt158	6	3.49	0.79	0.71	0.66	227-243
21	CUPVSiirt171	2	1.35	0.22	0.26	0.22	149-161
22	CUPVSiirt186	7	2.57	0.70	0.61	0.58	155-182
23	CUPVSiirt213	2	1.38	0.33	0.28	0.24	215-219
24	CUPVSiirt230	4	2.23	0.25	0.55	0.51	185-195
25	CUPVSiirt238	6	2.69	0.46	0.63	0.59	173-195

Table 4.4. Continued

26	CUPVSiirt242	8	3.21	0.53	0.69	0.66	138-167
27	CUPVSiirt243	3	1.88	0.38	0.47	0.41	142-146
28	CUPVSiirt256	5	2.76	0.46	0.64	0.58	171-187
29	CUPVSiirt259	5	2.22	0.71	0.55	0.52	200-218
30	CUPVSiirt265	2	1.78	0.13	0.44	0.34	194-198
31	CUPVSiirt271	5	3.37	0.78	0.70	0.65	98-114
32	CUPVSiirt284	3	2.18	0.42	0.54	0.46	220-243
33	CUPVSiirt294	5	2.65	0.38	0.62	0.56	117-128
34	CUPVSiirt297	4	2.42	0.50	0.59	0.50	136-146
35	CUPVSiirt298	6	3.40	0.71	0.71	0.67	121-133
36	CUPVSiirt308	5	3.10	0.35	0.68	0.62	167-175
37	CUPVSiirt312	2	1.18	0.17	0.15	0.14	164-166
38	CUPVSiirt316	5	2.70	0.38	0.63	0.59	254-280
39	CUPVSiirt320	2	1.97	0.88	0.49	0.37	173-179
40	CUPVSiirt328	4	2.00	0.41	0.50	0.47	186-196
41	CUPVSiirt333	4	2.19	0.32	0.54	0.46	144-150
42	CUPVSiirt340	6	3.36	0.58	0.70	0.66	129-163
43	CUPVSiirt343	4	1.96	0.33	0.49	0.43	121-127
44	CUPVSiirt349	4	3.59	0.58	0.72	0.67	174-190
45	CUPVSiirt357	9	5.60	0.77	0.82	0.80	193-231
46	CUPVSiirt358	9	5.80	0.27	0.83	0.81	215-269
47	CUPVSiirt368	3	1.40	0.05	0.28	0.26	153-161
48	CUPVSiirt415	3	2.43	0.30	0.59	0.52	158-170
49	CUPVSiirt436	5	2.32	0.83	0.57	0.51	92-110
50	CUPVSiirt446	2	1.99	0.42	0.50	0.37	236-242
51	CUPVSiirt465	9	5.12	0.54	0.80	0.78	143-167
52	CUPVSiirt472	3	1.79	0.42	0.44	0.38	172-185
53	CUPVSiirt476	4	2.59	0.58	0.61	0.57	162-171
54	CUPVSiirt479	5	2.13	0.61	0.53	0.49	165-192
55	CUPVSiirt496	4	1.69	0.32	0.41	0.37	170-177

Table 4.4. Continued

56	CUPVSiirt501	3	1.58	0.29	0.37	0.32	145-158
57	CUPVSiirt505	2	1.55	0.29	0.35	0.29	167-173
58	CUPVSiirt509	2	1.25	0.23	0.20	0.18	170-180
59	CUPVSiirt543	4	3.03	0.71	0.67	0.61	136-145
60	CUPVSiirt565	6	3.14	0.41	0.68	0.64	151-164
61	CUPVSiirt568x	3	1.29	0.17	0.23	0.21	100-103
62	CUPVSiirt568y	10	4.77	0.55	0.79	0.77	121-145
63	CUPVSiirt569	3	2.23	0.83	0.55	0.48	101-109
64	CUPVSiirt598	5	4.19	0.63	0.76	0.72	169-190
65	CUPVSiirt600	4	2.44	0.63	0.59	0.50	229-241
66	CUPVSiirt616	9	7.6	0.71	0.87	0.85	172-204
67	CUPVSiirt617	3	2.17	0.50	0.54	0.43	277-290
68	CUPVSiirt621	5	1.64	0.42	0.39	0.37	93-129
69	CUPVSiirt625	7	4.25	0.41	0.76	0.73	159-175
70	CUPVSiirt649	2	1.98	0.48	0.50	0.37	158-165
71	CUPVSiirt660	3	1.94	0.58	0.48	0.39	135-139
72	CUPVSiirt661	4	1.69	0.32	0.41	0.37	253-260
73	CUPVSiirt674	3	1.69	0.32	0.41	0.34	245-249
74	CUPVSiirt689x	4	2.07	0.43	0.52	0.48	200-208
75	CUPVSiirt689y	4	2.49	0.55	0.60	0.51	306-314
76	CUPVSiirt690	2	1.55	0.21	0.35	0.29	215-221
77	CUPVSiirt712	3	2.18	0.50	0.54	0.46	182-192
78	CUPVSiirt715	3	1.18	0.17	0.16	0.15	158-170
79	CUPVSiirt719	6	3.16	0.87	0.68	0.65	206-221
80	CUPVSiirt724	2	1.80	0.42	0.44	0.35	135-138
81	CUPVSiirt743	7	3.27	0.57	0.69	0.65	181-205
82	CUPVSiirt764	6	3.33	0.83	0.70	0.66	164-206
83	CUPVSiirt768	3	1.95	0.67	0.49	0.42	206-220
84	CUPVSiirt782	6	4.37	0.30	0.77	0.74	172-197
85	CUPVSiirt788	5	2.85	0.38	0.65	0.59	222-238

Table 4.4. Continued

86	CUPVSiirt794	5	2.98	0.67	0.66	0.63	216-244
87	CUPVSiirt796	5	3.87	0.62	0.74	0.70	104-130
88	CUPVSiirt803	4	2.05	0.29	0.51	0.47	202-106
89	CUPVSiirt818	7	3.07	0.50	0.67	0.64	171-185
90	CUPVSiirt838	5	2.60	0.86	0.61	0.55	159-181
91	CUPVSiirt841	6	3.14	0.48	0.68	0.64	163-186
92	CUPVSiirt847	7	4.26	0.62	0.77	0.73	226-256
93	CUPVSiirt855	6	3.27	0.70	0.69	0.66	248-274
94	CUPVSiirt858	7	3.81	0.85	0.74	0.70	167-198
95	CUPVSiirt875	3	2.03	0.17	0.51	0.42	157-165
96	CUPVSiirt876	7	4.80	0.96	0.79	0.76	185-207
97	CUPVSiirt883	5	1.89	0.29	0.47	0.44	172-188
98	CUPVSiirt889	6	3.02	0.58	0.67	0.61	168-188
99	CUPVSiirt891	3	1.83	0.30	0.45	0.37	142-161
100	CUPVSiirt907	3	2.36	0.57	0.58	0.48	92-98
101	CUPVSiirt929	6	2.26	0.46	0.56	0.52	85-101
102	CUPVSiirt931	5	2.61	0.21	0.62	0.55	146-160
103	CUPVSiirt932	7	4.25	0.65	0.76	0.73	123-183
104	CUPVSiirt949	5	1.97	0.38	0.49	0.46	160-197
105	CUPVSiirt951	2	1.13	0.13	0.12	0.11	164-167
106	CUPVSiirt956	10	2.63	0.43	0.62	0.60	133-200
107	CUPVSiirt961	5	2.51	0.21	0.60	0.54	201-210
108	CUPVSiirt975	3	1.61	0.38	0.38	0.34	145-154
109	CUPVSiirt986	3	2.8	0.46	0.64	0.57	157-169
110	CUPVSiirt989	5	1.89	0.33	0.47	0.44	148-176
111	CUPVSiirt1003	4	3.02	0.55	0.67	0.61	81-112
112	CUPVSiirt1008	4	1.87	0.35	0.47	0.42	165-174
113	CUPVSiirt1017	6	2.43	0.42	0.59	0.54	218-236
114	CUPVSiirt1021	3	2.23	0.71	0.55	0.47	127-135
115	CUPVSiirt1041	4	2.61	0.58	0.62	0.55	107-114

Table 4.4. Continued

116	CUPVSiirt1043	3	1.23	0.21	0.19	0.18	104-134
117	CUPVSiirt1047	3	1.40	0.00	0.29	0.26	140-148
118	CUPVSiirt1053	2	1.84	0.38	0.46	0.35	159-165
119	CUPVSiirt1055	2	1.18	0.17	0.15	0.14	155-169
120	CUPVSiirt1057	6	3.33	0.50	0.70	0.65	237-254
121	CUPVSiirt1062	3	2.18	0.50	0.54	0.46	139-144
122	CUPVSiirt1071	6	2.91	0.55	0.66	0.59	146-169
123	CUPVSiirt1092	4	3.12	0.75	0.68	0.62	149-155
124	CUPVSiirt1095	7	5.28	0.88	0.81	0.79	248-290
125	CUPVSiirt1116	6	2.13	0.54	0.53	0.49	158-191
126	CUPVSiirt1117	8	4.13	0.71	0.76	0.72	158-172
127	CUPVSiirt1120	5	2.02	0.58	0.50	0.46	198-208
128	CUPVSiirt1122	4	3.42	0.54	0.71	0.66	208-214
129	CUPVSiirt1127	6	2.37	0.54	0.58	0.55	120-134
130	CUPVSiirt1140	4	2.71	0.35	0.63	0.56	162-176
131	CUPVSiirt1145	5	2.81	0.57	0.64	0.59	158-173
132	CUPVSiirt1153	3	2.07	0.08	0.52	0.42	194-201
133	CUPVSiirt1163	5	3.24	0.68	0.69	0.64	156-166
134	CUPVSiirt1171	2	1.14	0.13	0.12	0.11	251-252
135	CUPVSiirt1182	4	2.32	0.54	0.57	0.52	167-179
136	CUPVSiirt1183	2	1.91	0.52	0.48	0.36	244-250
137	CUPVSiirt1188	2	1.57	0.48	0.36	0.30	158-168
138	CUPVSiirt1189	3	1.94	0.38	0.48	0.40	259-267
139	CUPVSiirt1191	4	3.07	0.63	0.67	0.63	158-190
140	CUPVSiirt1202	5	2.32	0.29	0.57	0.52	176-192
141	CUPVSiirt1214	3	1.47	0.29	0.32	0.29	160-164
142	CUPVSiirt1224	5	4.32	0.80	0.77	0.73	257-275
143	CUPVSiirt1238	3	1.53	0.42	0.35	0.32	239-252
144	CUPVSiirt1243	6	2.99	0.26	0.67	0.61	144-156
145	CUPVSiirt1250	6	2.53	0.54	0.60	0.55	175-189

Table 4.4. Continued

146	CUPVSiirt1260	4	1.29	0.25	0.23	0.21	166-175
147	CUPVSiirt1267	3	2.06	0.57	0.52	0.44	137-140
148	CUPVSiirt1271	4	1.30	0.25	0.23	0.22	223-232
149	CUPVSiirt1273	2	2.00	1.00	0.50	0.38	140-160
150	CUPVSiirt1278	2	1.55	0.13	0.35	0.29	176-181
151	CUPVSiirt1322	5	2.00	0.55	0.50	0.47	218-237
152	CUPVSiirt1326	5	3.26	0.55	0.69	0.65	189-198
153	CUPVSiirt1330	11	6.26	0.74	0.84	0.82	159-185
154	CUPVSiirt1331	3	1.90	0.38	0.47	0.42	104-115
155	CUPVSiirt1345	2	1.65	0.38	0.39	0.32	185-191
156	CUPVSiirt1353	8	2.78	0.73	0.64	0.62	173-197
157	CUPVSiirt1360	5	3.32	0.46	0.70	0.66	127-135
158	CUPVSiirt1372	5	1.92	0.09	0.48	0.45	182-194
159	CUPVSiirt1378	3	2.80	0.21	0.64	0.57	95-97
160	CUPVSiirt1388	3	2.28	0.65	0.56	0.47	183-201
161	CUPVSiirt1394	4	2.31	0.38	0.57	0.49	246-266
162	CUPVSiirt1399	2	1.09	0.08	0.08	0.08	183-193
163	CUPVSiirt1400	3	2.48	0.61	0.60	0.51	158-168
164	CUPVSiirt1402	4	2.83	0.54	0.65	0.60	178-187
165	CUPVSiirt1405	6	3.72	0.58	0.73	0.69	179-211
166	CUPVSiirt1406	3	2.07	0.17	0.52	0.44	172-191
167	CUPVSiirt1413	2	1.98	0.39	0.50	0.37	172-176
168	CUPVSiirt1417	6	2.36	0.43	0.58	0.55	138-176
169	CUPVSiirt1418	10	3.45	0.52	0.71	0.68	131-159
170	CUPVSiirt1431	3	1.60	0.18	0.38	0.34	209-215
171	CUPVSiirt1438	4	1.87	0.13	0.46	0.43	271-280
172	CUPVSiirt1442	4	1.42	0.24	0.29	0.28	110-118
173	CUPVSiirt1457	5	3.95	0.46	0.75	0.71	157-181
174	CUPVSiirt1477	5	2.59	0.48	0.61	0.57	109-126
175	CUPVSiirt1478	8	4.04	0.54	0.75	0.72	92-119

Table 4.4. Continued

176	CUPVSiirt1517	5	1.94	0.22	0.48	0.45	213-225
177	CUPVSiirt1547	2	1.04	0.04	0.04	0.04	120-126
178	CUPVSiirt1564	5	3.26	0.26	0.69	0.63	211-218
179	CUPVSiirt1567	5	2.67	0.58	0.63	0.58	195-203
180	CUPVSiirt1599	6	4.00	0.55	0.75	0.72	320-345
181	CUPVSiirt1611	2	1.18	0.17	0.15	0.14	194-205
182	CUPVSiirt1626	4	2.78	0.45	0.64	0.57	134-144
183	CUPVSiirt1628	4	1.87	0.42	0.46	0.43	125-146
184	CUPVSiirt1639	4	2.67	0.46	0.63	0.57	168-194
185	CUPVSiirt1640	5	1.63	0.13	0.39	0.36	161-176
186	CUPVSiirt1652	7	2.92	0.75	0.66	0.62	174-193
187	CUPVSiirt1655	3	2.28	0.57	0.56	0.47	150-156
188	CUPVSiirt1658	2	1.35	0.30	0.26	0.22	130-136
189	CUPVSiirt1667	5	2.26	0.45	0.56	0.49	170-186
190	CUPVSiirt1688	5	3.18	0.78	0.69	0.63	168-175
191	CUPVSiirt1734	4	2.19	0.54	0.54	0.49	168-178
192	CUPVSiirt1740	3	2.34	0.46	0.57	0.51	162-174
193	CUPVSiirt1742	7	2.94	0.41	0.66	0.62	181-213
194	CUPVSiirt1749	4	2.68	0.57	0.63	0.55	145-164
195	CUPVSiirt1759	4	3.39	0.58	0.70	0.65	139-159
196	CUPVSiirt1764	5	2.50	0.54	0.60	0.56	154-178
197	CUPVSiirt1768	4	1.66	0.48	0.40	0.37	121-128
198	CUPVSiirt1784	4	3.13	0.58	0.68	0.62	194-202
199	CUPVSiirt1788	2	1.18	0.17	0.15	0.14	183-189
200	CUPVSiirt1797	3	1.79	0.54	0.44	0.36	155-169
	Total	**897**	**-**	**-**	**-**	**-**	
	Mean	**4.5**	**2.57**	**0.46**	**0.55**	**0.50**	**81-345**

In *P. atlantica*, 200 SSR loci generated amplification products with a high rate of transferability (97.1%). Thirty-nine (19.5%) of the amplified SSR loci were monomorphic and the rest were polymorphic (80.5%). A total of 527 alleles were produced by 161 polymorphic SSR loci, ranging from 2 to 7 per locus an average of 3.3 alleles per locus. The highest number of alleles was obtained from the CUPVSiirt349, CUPVSiirt841 and CUPVSiirt1400 loci. The effective number of alleles ranged from 1.28 (CUPVSiirt18) to 6.4 (CUPVSiirt349, CUPVSiirt841, CUPVSiirt1400) with an average of *Ne* = 2.59. The observed heterozygosity (*Ho*) ranged from 0.0 to 1.00 with an average of *Ho* = 0.48. The CUPVSiirt86, CUPVSiirt129, CUPVSiirt333, CUPVSiirt569, CUPVSiirt661, CUPVSiirt712, CUPVSiirt719, CUPVSiirt768, CUPVSiirt782, CUPVSiirt838, CUPVSiirt841, CUPVSiirt1017, CUPVSiirt1047, CUPVSiirt1092, CUPVSiirt1326, CUPVSiirt1399, CUPVSiirt1400, CUPVSiirt1405, CUPVSiirt1639, CUPVSiirt1688 loci had the most heterozygous value (*Ho*=1). The average He value was 0.56, which ranged from 0.22 to 0.84. The most informative loci were obtained from CUPVSiirt349, CUPVSiirt841 and CUPVSiirt1400. The *PIC* values ranged from 0.19 to 0.82, with an average of 0.49. The highest PIC value was obtained in CUPVSiirt349, CUPVSiirt841, CUPVSiirt1400 loci. The CUPVSiirt465 and CUPVSiirt1092 loci also had high polymorphisim information content (*PIC* = 0.79) in *P. atlantica* (Table 4. 5).

Table 4.5. Number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), PIC values and allele ranges of 161 polymorphic SSR loci in *P. atlantica*.

No	Loci	*Na*	*Ne*	*Ho*	*He*	*PIC*	Allele Range (bp)
1	CUPVSiirt15	3	2.46	0.50	0.59	0.51	104-108
2	CUPVSiirt22	3	2.46	0.25	0.59	0.51	161-174
3	CUPVSiirt26	2	1.28	0.25	0.22	0.19	176-188
4	CUPVSiirt37	4	2.91	0.75	0.66	0.60	140-163
5	CUPVSiirt71	2	2.00	0.00	0.50	0.38	164-166
6	CUPVSiirt76	3	2.13	0.75	0.53	0.47	170-176
7	CUPVSiirt86	4	3.56	1.00	0.72	0.67	128-155
8	CUPVSiirt115	2	1.60	0.50	0.38	0.30	166-171
9	CUPVSiirt129	5	4.00	1.00	0.75	0.71	147-172
10	CUPVSiirt131	2	1.60	0.50	0.38	0.30	163-169
11	CUPVSiirt140	2	1.60	0.50	0.38	0.30	229-231
12	CUPVSiirt149	5	4.00	0.50	0.75	0.71	105-136
13	CUPVSiirt151	2	1.28	0.25	0.22	0.19	154-162
14	CUPVSiirt158	2	1.28	0.25	0.22	0.19	221-229
15	CUPVSiirt186	5	4.00	0.75	0.75	0.71	140-179
16	CUPVSiirt230	3	2.67	0.00	0.63	0.55	191-216
17	CUPVSiirt243	5	3.20	0.50	0.69	0.65	142-149
18	CUPVSiirt256	3	1.68	0.25	0.41	0.37	177-199
19	CUPVSiirt259	3	2.46	0.50	0.59	0.51	186-195
20	CUPVSiirt265	2	1.28	0.25	0.22	0.19	194-196
21	CUPVSiirt271	3	1.68	0.50	0.41	0.37	102-110
22	CUPVSiirt284	2	1.60	0.00	0.38	0.30	229-235
23	CUPVSiirt294	2	1.60	0.00	0.38	0.30	124-138
24	CUPVSiirt298	4	2.91	0.25	0.66	0.60	154-166
25	CUPVSiirt308	3	1.68	0.50	0.41	0.37	166-168

Table 4.5. Continued

26	CUPVSiirt312	3	2.13	0.75	0.53	0.47	154-172
27	CUPVSiirt316	4	2.91	0.25	0.66	0.60	259-280
28	CUPVSiirt328	3	2.00	0.33	0.50	0.45	181-204
29	CUPVSiirt333	4	2.91	1.00	0.66	0.60	144-153
30	CUPVSiirt340	5	4.00	0.50	0.75	0.71	129-171
31	CUPVSiirt349	7	6.40	0.75	0.84	0.82	172-199
32	CUPVSiirt357	3	2.13	0.50	0.53	0.47	193-202
33	CUPVSiirt368	3	2.67	0.00	0.63	0.55	166-188
34	CUPVSiirt415	2	1.28	0.25	0.22	0.19	145-153
35	CUPVSiirt436	4	3.56	0.25	0.72	0.67	92-114
36	CUPVSiirt446	3	2.13	0.25	0.53	0.47	230-242
37	CUPVSiirt465	6	5.33	0.50	0.81	0.79	145-178
38	CUPVSiirt472	3	1.68	0.25	0.41	0.37	294-325
39	CUPVSiirt476	2	1.28	0.25	0.22	0.19	157-162
40	CUPVSiirt479	2	1.38	0.33	0.28	0.24	165-187
41	CUPVSiirt496	2	1.60	0.00	0.38	0.30	181-199
42	CUPVSiirt505	2	1.60	0.50	0.38	0.30	175-179
43	CUPVSiirt509	4	2.29	0.50	0.56	0.52	162-181
44	CUPVSiirt543	4	2.91	0.75	0.66	0.60	132-142
45	CUPVSiirt565	2	1.28	0.25	0.22	0.19	140-142
46	CUPVSiirt568y	4	3.60	0.70	0.70	0.67	123-126
47	CUPVSiirt569	3	2.67	1.00	0.63	0.55	95-102
48	CUPVSiirt598	4	2.29	0.75	0.56	0.52	163-186
49	CUPVSiirt600	2	1.88	0.75	0.47	0.36	229-231
50	CUPVSiirt616	4	2.91	0.75	0.66	0.6	176-204
51	CUPVSiirt625	3	2.46	0.50	0.59	0.51	169-175
52	CUPVSiirt660	3	2.46	0.50	0.59	0.51	131-135
53	CUPVSiirt661	3	2.46	1.00	0.59	0.51	251-255
54	CUPVSiirt674	3	1.68	0.50	0.41	0.37	235-243
55	CUPVSiirt689x	2	1.80	0.00	0.44	0.35	200-210

Table 4.5. Continued

56	CUPVSiirt689y	2	1.88	0.75	0.47	0.36	306-314
57	CUPVSiirt712	5	4.00	1.00	0.75	0.71	180-198
58	CUPVSiirt715	4	2.29	0.50	0.56	0.52	160-176
59	CUPVSiirt719	4	3.20	1.00	0.69	0.63	209-231
60	CUPVSiirt742	3	2.91	0.50	0.66	0.58	201-205
61	CUPVSiirt743	3	1.68	0.25	0.41	0.37	163-167
62	CUPVSiirt764	2	1.88	0.25	0.47	0.36	164-176
63	CUPVSiirt768	3	2.46	1.00	0.59	0.51	206-218
64	CUPVSiirt782	4	2.91	1.00	0.66	0.60	176-188
65	CUPVSiirt788	3	2.46	0.50	0.59	0.51	214-220
66	CUPVSiirt794	3	1.68	0.25	0.41	0.37	216-238
67	CUPVSiirt796	4	2.91	0.50	0.66	0.60	114-127
68	CUPVSiirt818	2	1.28	0.25	0.22	0.19	165-181
69	CUPVSiirt836	3	2.67	0.00	0.63	0.55	164-166
70	CUPVSiirt838	5	4.57	1.00	0.78	0.75	159-171
71	CUPVSiirt841	7	6.40	1.00	0.84	0.82	155-185
72	CUPVSiirt858	2	1.28	0.25	0.22	0.19	177-183
73	CUPVSiirt875	5	4.00	0.75	0.75	0.71	157-177
74	CUPVSiirt876	3	2.46	0.50	0.59	0.51	183-187
75	CUPVSiirt891	2	1.28	0.25	0.22	0.19	142-148
76	CUPVSiirt907	2	1.28	0.25	0.22	0.19	92-95
77	CUPVSiirt924	2	1.60	0.00	0.38	0.30	87-89
78	CUPVSiirt929	4	2.91	0.25	0.66	0.60	87-105
79	CUPVSiirt949	2	1.88	0.25	0.47	0.36	167-179
80	CUPVSiirt956	4	3.60	0.67	0.72	0.67	162-198
81	CUPVSiirt961	3	2.46	0.50	0.59	0.51	190-204
82	CUPVSiirt989	2	1.60	0.00	0.38	0.30	148-152
83	CUPVSiirt1003	3	2.00	0.33	0.50	0.45	81-102
84	CUPVSiirt1017	4	2.91	1.00	0.66	0.60	214-245
85	CUPVSiirt1021	2	1.88	0.75	0.47	0.36	125-131

Table 4.5. Continued

86	CUPVSiirt1043	4	3.20	0.75	0.69	0.63	113-147
87	CUPVSiirt1047	3	2.67	1.00	0.63	0.55	127-157
88	CUPVSiirt1053	3	2.13	0.25	0.53	0.47	178-189
89	CUPVSiirt1055	3	2.91	0.50	0.66	0.58	155-169
90	CUPVSiirt1057	5	4.00	0.75	0.75	0.71	230-258
91	CUPVSiirt1062	5	4.00	0.75	0.75	0.71	138-146
92	CUPVSiirt1071	2	1.60	0.50	0.38	0.30	146-150
93	CUPVSiirt1092	6	5.33	1.00	0.81	0.79	145-163
94	CUPVSiirt1095	2	2.00	0.33	0.50	0.38	242-244
95	CUPVSiirt1116	5	3.20	0.75	0.69	0.65	151-170
96	CUPVSiirt1117	3	2.13	0.75	0.53	0.47	150-170
97	CUPVSiirt1120	3	2.13	0.50	0.53	0.47	198-218
98	CUPVSiirt1122	2	1.60	0.00	0.38	0.30	209-215
99	CUPVSiirt1140	2	1.88	0.25	0.47	0.36	162-166
100	CUPVSiirt1145	3	2.13	0.75	0.53	0.47	162-174
101	CUPVSiirt1153	2	1.88	0.25	0.47	0.36	192-195
102	CUPVSiirt1171	5	4.00	0.75	0.75	0.71	241-270
103	CUPVSiirt1182	4	2.91	0.75	0.66	0.60	161-173
104	CUPVSiirt1188	2	1.88	0.25	0.47	0.36	158-168
105	CUPVSiirt1189	2	1.88	0.25	0.47	0.36	247-249
106	CUPVSiirt1191	2	2.00	0.00	0.50	0.38	162-164
107	CUPVSiirt1202	4	2.29	0.50	0.56	0.52	174-182
108	CUPVSiirt1214	4	4.00	0.00	0.75	0.70	160-171
109	CUPVSiirt1224	3	2.13	0.25	0.53	0.47	257-265
110	CUPVSiirt1238	3	2.67	0.00	0.63	0.55	239-249
111	CUPVSiirt1243	2	1.60	0.00	0.38	0.30	138-142
112	CUPVSiirt1250	2	1.60	0.00	0.38	0.30	176-177
113	CUPVSiirt1260	4	2.91	0.75	0.66	0.60	157-172
114	CUPVSiirt1267	3	1.68	0.50	0.41	0.37	133-155
115	CUPVSiirt1271	2	1.60	0.50	0.38	0.30	221-223

Table 4.5. Continued

116	CUPVSiirt1273	3	1.68	0.50	0.41	0.37	140-154
117	CUPVSiirt1322	3	2.13	0.75	0.53	0.47	218-223
118	CUPVSiirt1326	5	4.57	1.00	0.78	0.75	192-211
119	CUPVSiirt1330	4	2.91	0.75	0.66	0.60	157-183
120	CUPVSiirt1331	5	4.57	0.75	0.78	0.75	110-119
121	CUPVSiirt1345	2	1.38	0.33	0.28	0.24	173-179
122	CUPVSiirt1372	3	2.13	0.50	0.53	0.47	112-130
123	CUPVSiirt1378	3	1.68	0.25	0.41	0.37	83-97
124	CUPVSiirt1394	4	3.60	0.33	0.72	0.67	234-270
125	CUPVSiirt1399	4	2.91	1.00	0.66	0.60	193-216
126	CUPVSiirt1400	7	6.40	1.00	0.84	0.82	168-186
127	CUPVSiirt1402	3	2.13	0.25	0.53	0.47	174-181
128	CUPVSiirt1405	5	4.57	1.00	0.78	0.75	176-206
129	CUPVSiirt1406	4	4.00	0.00	0.75	0.70	172-185
130	CUPVSiirt1413	3	1.68	0.50	0.41	0.37	166-172
131	CUPVSiirt1417	4	2.91	0.50	0.66	0.60	142-153
132	CUPVSiirt1418	2	1.88	0.25	0.47	0.36	155-157
133	CUPVSiirt1431	3	2.91	0.75	0.66	0.58	203-213
134	CUPVSiirt1438	4	2.91	0.50	0.66	0.60	265-290
135	CUPVSiirt1442	4	3.56	0.50	0.72	0.67	110-126
136	CUPVSiirt1457	3	2.67	0.00	0.63	0.55	159-195
137	CUPVSiirt1477	4	3.00	0.67	0.67	0.62	117-120
138	CUPVSiirt1478	4	3.56	0.25	0.72	0.67	84-117
139	CUPVSiirt1517	2	2.00	0.50	0.50	0.38	213-219
140	CUPVSiirt1547	2	1.88	0.75	0.47	0.36	120-126
141	CUPVSiirt1564	4	3.56	0.25	0.72	0.67	210-217
142	CUPVSiirt1567	4	3.56	0.50	0.72	0.67	190-195
143	CUPVSiirt1599	4	4.00	0.00	0.75	0.70	294-342
144	CUPVSiirt1611	5	4.57	0.25	0.78	0.75	194-215
145	CUPVSiirt1626	3	2.67	0.00	0.63	0.55	123-153

Table 4.5. Continued

146	CUPVSiirt1639	2	2.00	1.00	0.50	0.38	169-170
147	CUPVSiirt1640	4	3.56	0.75	0.72	0.67	146-180
148	CUPVSiirt1652	2	1.28	0.25	0.22	0.19	172-191
149	CUPVSiirt1655	5	3.20	0.75	0.69	0.65	180-213
150	CUPVSiirt1658	2	1.88	0.75	0.47	0.36	136-142
151	CUPVSiirt1667	3	2.46	0.25	0.59	0.51	164-168
152	CUPVSiirt1688	5	4.57	1.00	0.78	0.75	168-179
153	CUPVSiirt1705	3	2.67	0.00	0.63	0.55	231-251
154	CUPVSiirt1734	2	1.88	0.75	0.47	0.36	168-174
155	CUPVSiirt1740	3	1.68	0.50	0.41	0.37	162-174
156	CUPVSiirt1742	2	2.00	0.00	0.50	0.38	151-163
157	CUPVSiirt1749	3	2.46	0.50	0.59	0.51	145-158
158	CUPVSiirt1759	4	2.91	0.50	0.66	0.60	146-159
159	CUPVSiirt1768	3	2.91	0.25	0.66	0.58	121-126
160	CUPVSiirt1784	4	3.20	0.50	0.69	0.63	194-211
161	CUPVSiirt1797	3	2.13	0.50	0.53	0.47	155-161
	Total	**527**					
	Mean	**3.3**	**2.59**	**0.48**	**0.56**	**0.49**	**83-342**

In *P. integerrima*, the transferability of SSR loci was also high, with a rate of 93.7%. Of the amplified SSR loci, 157 (81.3%) were polymorphic in *P. integerrima*. A total of 416 alleles were produced by 157 SSR loci with an average of 2.70 alleles per locus, and the highest number (*Na* = 5) of alleles was obtained from the CUPVSiirt131, CUPVSiirt742, CUPVSiirt838, and CUPVSiirt1330 loci. The effective number of alleles ranged from 1.28 to 4.57 with an average of 2.21. The highest effective number of alleles (4.57) was calculated at the CUPVSiirt742 locus. The high effective numbers of alleles were also obtained in CUPVSiirt131, CUPVSiirt838 (*Ne* = 4) loci. The average observed (*Ho*) and expected (*He*) heterozygosities were 0.50 and 0.52, respectively. The expected heterozygosity

(*He*) values varied from 0.22 to 0.78 with an average of 0.55. The highest values for expected heterozygosity (0.78)) were produced from the CUPVSiirt742 locus. The CUPVSiirt131 and CUPVSiirt838 loci were also the most informative than the others due to their higher patterns and having *He* = 0.72. The polymorphism information content (*PIC*) values ranged between 0.19 and 0.75 and the highest *PIC* value was obtained from CUPVSiirt742 locus (0.75). The average *PIC* value in *P. integerrima* was 0.44 (Table 4. 6).

Table 4.6. Number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), PIC values and allele range of 157 polymorphic SSR loci in *P. integerrima*.

No	Loci	*Na*	*Ne*	*Ho*	*He*	*PIC*	Allele range (bp)
1	CUPVSiirt15	3	2.13	0.25	0.53	0.47	104-123
2	CUPVSiirt22	3	2.46	0.75	0.59	0.51	160-182
3	CUPVSiirt26	2	1.88	0.75	0.47	0.36	164-188
4	CUPVSiirt37	3	2.46	0.75	0.59	0.51	140-167
5	CUPVSiirt50	2	1.38	0.33	0.28	0.24	162-170
6	CUPVSiirt71	3	2.67	0.00	0.63	0.55	156-167
7	CUPVSiirt76	3	2.13	0.50	0.53	0.47	172-176
8	CUPVSiirt86	3	2.67	0.50	0.63	0.55	111-142
9	CUPVSiirt115	2	1.88	0.25	0.47	0.36	166-171
10	CUPVSiirt125	2	2.00	1.00	0.50	0.38	167-177
11	CUPVSiirt129	4	3.56	1.00	0.72	0.67	135-172
12	CUPVSiirt131	5	4.00	0.75	0.75	0.71	162-175
13	CUPVSiirt149	3	2.13	0.75	0.53	0.47	104-108
14	CUPVSiirt151	3	2.13	0.75	0.53	0.47	160-164
15	CUPVSiirt171	3	2.13	0.75	0.53	0.47	142-161
16	CUPVSiirt213	2	1.28	0.25	0.22	0.19	211-219

Table 4.6. Continued

17	CUPVSiirt238	4	2.91	0.50	0.66	0.60	171-191
18	CUPVSiirt242	2	1.28	0.25	0.22	0.19	134-155
19	CUPVSiirt243	3	2.91	0.75	0.66	0.58	140-149
20	CUPVSiirt256	2	1.60	0.00	0.38	0.30	179-183
21	CUPVSiirt259	3	2.46	1.00	0.59	0.51	186-210
22	CUPVSiirt265	2	1.88	0.75	0.47	0.36	194-200
23	CUPVSiirt271	2	2.00	0.00	0.50	0.38	101-104
24	CUPVSiirt284	3	2.46	1.00	0.59	0.51	235-247
25	CUPVSiirt294	3	1.68	0.50	0.41	0.37	118-138
26	CUPVSiirt297	2	1.60	0.00	0.38	0.30	138-139
27	CUPVSiirt298	4	2.29	0.50	0.56	0.52	116-152
28	CUPVSiirt308	3	3.00	0.67	0.67	0.59	166-173
29	CUPVSiirt316	2	1.60	0.00	0.38	0.30	262-264
30	CUPVSiirt333	3	2.91	0.75	0.66	0.58	144-153
31	CUPVSiirt340	2	2.00	0.50	0.50	0.38	129-157
32	CUPVSiirt349	2	1.60	0.00	0.38	0.30	172-174
33	CUPVSiirt357	3	2.46	0.50	0.59	0.51	188-202
34	CUPVSiirt436	3	2.13	0.25	0.53	0.47	91-108
35	CUPVSiirt446	3	2.46	1.00	0.59	0.51	221-242
36	CUPVSiirt465	2	1.60	0.00	0.38	0.30	145-150
37	CUPVSiirt476	3	1.68	0.50	0.41	0.37	149-165
38	CUPVSiirt496	2	1.60	0.00	0.38	0.30	189-193
39	CUPVSiirt501	3	2.91	0.25	0.66	0.58	146-158
40	CUPVSiirt505	3	2.91	0.75	0.66	0.58	175-186
41	CUPVSiirt509	3	2.00	0.33	0.50	0.45	164-180
42	CUPVSiirt543	3	2.46	1.00	0.59	0.51	119-149
43	CUPVSiirt565	3	2.13	0.75	0.53	0.47	134-152
44	CUPVSiirt568y	3	2.91	0.50	0.66	0.58	123-126
45	CUPVSiirt569	2	1.28	0.25	0.22	0.19	96-102
46	CUPVSiirt598	2	1.60	0.00	0.38	0.30	164-202

Table 4.6. Continued

47	CUPVSiirt600	2	1.88	0.75	0.47	0.36	227-231
48	CUPVSiirt616	2	2.00	0.00	0.50	0.38	178-204
49	CUPVSiirt617	2	1.80	0.00	0.44	0.35	281-290
50	CUPVSiirt621	4	3.56	1.00	0.72	0.67	93-100
51	CUPVSiirt625	4	3.20	0.75	0.69	0.63	165-175
52	CUPVSiirt649	2	2.00	1.00	0.50	0.38	158-165
53	CUPVSiirt660	3	2.91	0.75	0.66	0.58	131-135
54	CUPVSiirt661	2	2.00	1.00	0.50	0.38	251-288
55	CUPVSiirt689y	2	2.00	1.00	0.50	0.38	306-314
56	CUPVSiirt712	2	1.88	0.25	0.47	0.36	190-204
57	CUPVSiirt715	3	2.13	0.50	0.53	0.47	139-143
58	CUPVSiirt719	2	2.00	0.00	0.50	0.38	221-231
59	CUPVSiirt724	2	2.00	1.00	0.50	0.38	132-135
60	CUPVSiirt742	5	4.57	1.00	0.78	0.75	196-209
61	CUPVSiirt764	2	2.00	1.00	0.50	0.38	164-176
62	CUPVSiirt768	2	2.00	0.00	0.50	0.38	206-220
63	CUPVSiirt782	4	2.29	0.50	0.56	0.52	176-188
64	CUPVSiirt788	2	2.00	0.00	0.50	0.38	214-216
65	CUPVSiirt794	4	3.20	1.00	0.69	0.63	212-232
66	CUPVSiirt796	3	2.13	0.75	0.53	0.47	104-116
67	CUPVSiirt803	3	2.67	0.50	0.63	0.55	202-206
68	CUPVSiirt818	3	1.68	0.50	0.41	0.37	171-177
69	CUPVSiirt836	3	2.67	0.50	0.63	0.55	164-168
70	CUPVSiirt838	5	4.00	1.00	0.75	0.71	149-163
71	CUPVSiirt841	4	3.56	0.75	0.72	0.67	157-177
72	CUPVSiirt855	2	2.00	0.00	0.50	0.38	248-250
73	CUPVSiirt858	3	2.91	0.75	0.66	0.58	175-198
74	CUPVSiirt875	3	2.67	0.50	0.63	0.55	163-169
75	CUPVSiirt876	2	1.60	0.00	0.38	0.30	187-193
76	CUPVSiirt883	2	2.00	0.00	0.50	0.38	174-185

Table 4.6. Continued

77	CUPVSiirt889	2	1.88	0.75	0.47	0.36	168-181
78	CUPVSiirt891	2	2.00	0.50	0.50	0.38	142-148
79	CUPVSiirt907	2	2.00	0.00	0.50	0.38	92-95
80	CUPVSiirt924	2	2.00	0.00	0.50	0.38	84-87
81	CUPVSiirt929	2	1.88	0.25	0.47	0.36	83-103
82	CUPVSiirt949	2	2.00	1.00	0.50	0.38	167-179
83	CUPVSiirt956	4	3.56	1.00	0.72	0.67	105-150
84	CUPVSiirt961	2	1.88	0.75	0.47	0.36	190-192
85	CUPVSiirt975	2	2.00	1.00	0.50	0.38	142-145
86	CUPVSiirt989	3	1.68	0.50	0.41	0.37	152-164
87	CUPVSiirt1003	2	2.00	0.00	0.50	0.38	71-75
88	CUPVSiirt1017	2	1.88	0.75	0.47	0.36	205-245
89	CUPVSiirt1021	3	2.46	0.25	0.59	0.51	123-131
90	CUPVSiirt1041	3	2.13	0.75	0.53	0.47	103-107
91	CUPVSiirt1043	2	1.60	0.50	0.38	0.30	89-127
92	CUPVSiirt1047	2	2.00	1.00	0.50	0.38	125-133
93	CUPVSiirt1055	2	2.00	0.33	0.50	0.38	157-163
94	CUPVSiirt1057	2	1.60	0.00	0.38	0.30	237-252
95	CUPVSiirt1071	2	2.00	1.00	0.50	0.38	123-133
96	CUPVSiirt1092	3	3.00	0.67	0.67	0.59	151-165
97	CUPVSiirt1095	2	1.80	0.00	0.44	0.35	240-242
98	CUPVSiirt1116	2	1.88	0.75	0.47	0.36	151-162
99	CUPVSiirt1117	2	2.00	0.50	0.50	0.38	150-158
100	CUPVSiirt1120	2	2.00	0.00	0.50	0.38	197-198
101	CUPVSiirt1127	3	2.91	0.75	0.66	0.58	118-134
102	CUPVSiirt1140	2	2.00	1.00	0.50	0.38	157-162
103	CUPVSiirt1145	2	1.60	0.50	0.38	0.30	154-164
104	CUPVSiirt1163	2	1.38	0.33	0.28	0.24	140-152
105	CUPVSiirt1171	3	2.13	0.75	0.53	0.47	230-255
106	CUPVSiirt1182	3	2.13	0.50	0.53	0.47	155-179

Table 4.6. Continued

107	CUPVSiirt1183	2	1.88	0.25	0.47	0.36	235-237
108	CUPVSiirt1191	2	2.00	0.50	0.50	0.38	162-164
109	CUPVSiirt1202	3	2.46	0.50	0.59	0.51	178-186
110	CUPVSiirt1214	2	1.60	0.00	0.38	0.30	180-182
111	CUPVSiirt1224	2	1.60	0.00	0.38	0.30	257-273
112	CUPVSiirt1238	4	3.20	1.00	0.69	0.63	233-255
113	CUPVSiirt1243	4	3.60	0.33	0.72	0.67	138-150
114	CUPVSiirt1250	3	2.67	1.00	0.63	0.55	176-192
115	CUPVSiirt1267	2	2.00	1.00	0.50	0.38	133-149
116	CUPVSiirt1273	2	2.00	1.00	0.50	0.38	140-154
117	CUPVSiirt1278	3	2.67	1.00	0.63	0.55	176-181
118	CUPVSiirt1322	4	3.56	0.25	0.72	0.67	218-226
119	CUPVSiirt1326	2	1.28	0.25	0.22	0.19	186-211
120	CUPVSiirt1330	5	3.20	0.75	0.69	0.65	157-185
121	CUPVSiirt1345	3	2.46	0.25	0.59	0.51	173-191
122	CUPVSiirt1353	2	1.28	0.25	0.22	0.19	169-197
123	CUPVSiirt1372	4	2.29	0.50	0.56	0.52	112-129
124	CUPVSiirt1378	2	1.88	0.25	0.47	0.36	84-97
125	CUPVSiirt1388	3	2.13	0.75	0.53	0.47	177-183
126	CUPVSiirt1400	3	2.67	1.00	0.63	0.55	166-172
127	CUPVSiirt1402	3	2.91	0.25	0.66	0.58	174-181
128	CUPVSiirt1405	2	1.38	0.33	0.28	0.24	185-219
129	CUPVSiirt1406	2	1.60	0.00	0.38	0.30	196-197
130	CUPVSiirt1413	2	2.00	0.75	0.50	0.51	172-190
131	CUPVSiirt1418	2	2.00	0.00	0.50	0.38	144-153
132	CUPVSiirt1431	2	1.88	0.25	0.47	0.36	209-213
133	CUPVSiirt1438	2	2.00	0.00	0.50	0.38	277-280
134	CUPVSiirt1442	2	1.88	0.25	0.47	0.36	122-128
135	CUPVSiirt1457	3	2.46	0.50	0.59	0.51	189-199
136	CUPVSiirt1477	3	2.13	0.75	0.53	0.47	115-119

Table 4.6. Continuesd

137	CUPVSiirt1517	3	2.46	0.50	0.59	0.51	213-221
138	CUPVSiirt1547	2	1.60	0.50	0.38	0.30	120-126
139	CUPVSiirt1564	3	2.13	0.50	0.53	0.47	208-216
140	CUPVSiirt1567	3	1.68	0.50	0.41	0.37	191-215
141	CUPVSiirt1611	2	1.88	0.25	0.47	0.36	193-205
142	CUPVSiirt1626	2	1.28	0.25	0.22	0.19	124-125
143	CUPVSiirt1639	2	2.00	0.00	0.50	0.38	170-176
144	CUPVSiirt1640	3	2.67	0.50	0.63	0.55	144-174
145	CUPVSiirt1652	3	2.46	0.50	0.59	0.51	172-178
146	CUPVSiirt1655	3	2.13	0.75	0.53	0.47	158-192
147	CUPVSiirt1658	4	3.20	0.75	0.69	0.63	136-167
148	CUPVSiirt1667	3	2.91	0.25	0.66	0.58	164-170
149	CUPVSiirt1705	2	1.60	0.50	0.38	0.30	241-251
150	CUPVSiirt1714	2	1.38	0.33	0.28	0.24	196-207
151	CUPVSiirt1740	2	1.28	0.25	0.22	0.19	162-168
152	CUPVSiirt1742	2	1.28	0.25	0.22	0.19	179-207
153	CUPVSiirt1749	4	3.56	0.75	0.72	0.67	145-154
154	CUPVSiirt1759	4	3.00	1.00	0.67	0.62	151-159
155	CUPVSiirt1768	2	2.00	0.00	0.50	0.38	123-128
156	CUPVSiirt1784	3	1.68	0.50	0.41	0.37	193-205
157	CUPVSiirt1797	3	2.91	0.25	0.66	0.58	155-159
	Total	**416**					
	Mean	**2.7**	**2.21**	**0.50**	**0.52**	**0.44**	**71- 290**

In *P. terebinthus*, 183 SSR loci (88.8%) generated amplification products and 142 (77.6%) were polymorphic. A total of 416 alleles were produced by 142 polymorphic SSR loci. Number of alleles (*Na*) ranged from 1 to 7 with an average of 3.4 alleles per locus and the highest number of allele (*Na* = 7) was obtained from the CUPVSiirt1017, CUPVSiirt1326, CUPVSiirt1405, CUPVSiirt1406 loci. The effective number of alleles ranged from 1.28 to 6.40. The highest Ne was obtained from CUPVSiirt1017, CUPVSiirt1326, CUPVSiirt1405, CUPVSiirt1406 loci. The observed heterozygosity (*Ho*) ranged from 0.0 to 1.0 with an average of 0.47. The expected heterozygosity (*He*) values varied from 0.22 to 0.84 with an average of 0.56. The CUPVSiirt1017, CUPVSiirt1326, CUPVSiirt1405, CUPVSiirt1406 loci were more informative than the others. The polymorphic information content (*PIC*) values changed between 0.19 and 0.82 with an average of 0.50, and the highest (*PIC*) values were obtained from CUPVSiirt1017, CUPVSiirt1326, CUPVSiirt1405, CUPVSiirt1406 loci (Table 4.7).

In *P. terebinthus*, the highest number of alleles (*Na* = 7), effective number of alleles (*Ne* = 6.4), expected heterozygosity (0.84), and PIC (0.82) were obtained from the CUPVSiirt1017, CUPVSiirt1326, CUPVSiirt1405, and CUPVSiirt1406 loci. The average *He* and *PIC* values were 0.56 and 0.50, respectively (Table 4.7).

Table 4.7. Number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), PIC values and allele range of 142 polymorphic SSR loci in *P. terebinthus*.

No	Loci	*Na*	*Ne*	*Ho*	*He*	*PIC*	Allele range (bp)
1	CUPVSiirt15	3	2.67	0.00	0.63	0.55	96-104
2	CUPVSiirt22	2	1.88	0.75	0.47	0.36	160-181
3	CUPVSiirt26	2	1.28	0.25	0.22	0.19	170-179
4	CUPVSiirt37	2	2.00	0.00	0.50	0.38	148-164
5	CUPVSiirt50	5	4.57	0.50	0.78	0.75	161-184
6	CUPVSiirt71	2	1.80	0.00	0.44	0.35	132-134
7	CUPVSiirt76	2	1.28	0.25	0.22	0.19	172-174
8	CUPVSiirt86	4	3.56	0.75	0.72	0.67	126-151
9	CUPVSiirt95	4	2.91	0.25	0.66	0.6	191-235
10	CUPVSiirt121	3	2.46	0.25	0.59	0.51	121-127
11	CUPVSiirt125	5	4.57	0.75	0.78	0.75	175-203
12	CUPVSiirt129	4	2.29	0.50	0.56	0.52	143-172
13	CUPVSiirt131	3	2.46	0.50	0.59	0.51	156-170
14	CUPVSiirt149	3	1.68	0.50	0.41	0.37	103-106
15	CUPVSiirt151	3	1.68	0.25	0.41	0.37	150-168
16	CUPVSiirt186	2	1.28	0.25	0.22	0.19	160-170
17	CUPVSiirt213	3	1.68	0.50	0.41	0.37	297-211
18	CUPVSiirt230	3	2.91	0.50	0.66	0.58	177-186
19	CUPVSiirt242	2	1.88	0.25	0.47	0.36	134-136
20	CUPVSiirt243	6	5.33	0.75	0.81	0.79	144-166
21	CUPVSiirt256	2	2.00	0.00	0.50	0.38	177-183
22	CUPVSiirt259	3	2.46	0.50	0.59	0.51	194-198
23	CUPVSiirt271	4	2.91	0.75	0.66	0.6	96-110
24	CUPVSiirt284	2	2.00	0.00	0.50	0.38	239-248

Table 4.7. Continued

25	CUPVSiirt294	2	1.28	0.25	0.22	0.19	116-118
26	CUPVSiirt297	3	2.13	0.25	0.53	0.47	139-141
27	CUPVSiirt298	5	4.00	1.00	0.75	0.71	106-198
28	CUPVSiirt308	3	2.67	0.50	0.63	0.55	166-173
29	CUPVSiirt312	4	3.56	0.75	0.72	0.67	154-168
30	CUPVSiirt349	3	2.67	1.00	0.63	0.55	168-179
31	CUPVSiirt357	3	1.68	0.50	0.41	0.37	185-192
32	CUPVSiirt436	4	3.56	0.25	0.72	0.67	92-118
33	CUPVSiirt465	5	4.00	0.75	0.75	0.71	143-177
34	CUPVSiirt472	3	2.13	0.25	0.53	0.47	206-237
35	CUPVSiirt476	2	1.28	0.25	0.22	0.19	146-149
36	CUPVSiirt479	2	1.38	0.33	0.28	0.24	165-187
37	CUPVSiirt496	2	2.00	0.00	0.50	0.38	158-163
38	CUPVSiirt501	3	2.67	0.50	0.63	0.55	153-171
39	CUPVSiirt505	4	3.56	0.75	0.72	0.67	154-186
40	CUPVSiirt509	2	1.28	0.25	0.22	0.19	175-180
41	CUPVSiirt543	3	2.13	0.50	0.53	0.47	119-140
42	CUPVSiirt565	6	5.33	0.50	0.81	0.79	138-154
43	CUPVSiirt568y	3	1.68	0.50	0.41	0.37	121-126
44	CUPVSiirt569	2	1.28	0.25	0.22	0.19	102-109
45	CUPVSiirt598	5	4.50	1.00	0.78	0.74	178-204
46	CUPVSiirt600	2	1.60	0.00	0.38	0.30	225-227
47	CUPVSiirt616	2	2.00	0.00	0.50	0.38	138-140
48	CUPVSiirt621	2	1.60	0.00	0.38	0.30	102-103
49	CUPVSiirt625	5	4.57	0.25	0.78	0.75	175-185
50	CUPVSiirt649	2	1.28	0.25	0.22	0.19	170-177
51	CUPVSiirt660	4	3.60	1.00	0.72	0.67	129-146
52	CUPVSiirt712	2	1.28	0.25	0.22	0.19	186-190
53	CUPVSiirt715	3	2.13	0.50	0.53	0.47	139-158

Table 4.7. Continued

54	CUPVSiirt719	3	2.57	1.00	0.61	0.54	212-234
55	CUPVSiirt742	6	5.33	1.00	0.81	0.79	198-208
56	CUPVSiirt768	3	2.13	0.75	0.53	0.47	210-220
57	CUPVSiirt782	2	1.60	0.50	0.38	0.30	176-188
58	CUPVSiirt788	6	5.33	1.00	0.81	0.79	210-248
59	CUPVSiirt794	5	4.00	0.75	0.75	0.71	216-230
60	CUPVSiirt796	6	4.57	1.00	0.78	0.75	92-115
61	CUPVSiirt803	2	1.88	0.75	0.47	0.36	258-266
62	CUPVSiirt818	3	2.13	0.50	0.53	0.47	177-183
63	CUPVSiirt836	5	3.20	0.75	0.69	0.65	148-162
64	CUPVSiirt838	6	5.33	0.75	0.81	0.79	149-170
65	CUPVSiirt841	5	4.50	0.67	0.78	0.74	146-171
66	CUPVSiirt855	2	1.60	0.50	0.38	0.30	248-252
67	CUPVSiirt858	2	1.28	0.25	0.22	0.19	175-177
68	CUPVSiirt875	5	4.00	0.50	0.75	0.71	165-190
69	CUPVSiirt876	6	5.33	1.00	0.81	0.79	191-221
70	CUPVSiirt883	5	4.00	0.50	0.75	0.71	179-208
71	CUPVSiirt889	2	1.60	0.00	0.38	0.30	177-181
72	CUPVSiirt891	3	1.68	0.50	0.41	0.37	142-168
73	CUPVSiirt929	2	1.88	0.75	0.47	0.36	83-85
74	CUPVSiirt949	2	1.28	0.25	0.22	0.19	167-171
75	CUPVSiirt951	3	2.67	0.00	0.63	0.55	154-184
76	CUPVSiirt956	2	2.00	0.00	0.50	0.38	131-141
77	CUPVSiirt975	2	1.60	0.00	0.38	0.30	139-140
78	CUPVSiirt986	4	2.29	0.75	0.56	0.52	157-165
79	CUPVSiirt989	5	4.00	0.75	0.75	0.71	140-168
80	CUPVSiirt1003	4	3.60	0.33	0.72	0.67	73-97
81	CUPVSiirt1008	2	1.60	0.00	0.38	0.30	165-168
82	CUPVSiirt1017	7	6.40	0.75	0.84	0.82	214-239

Table 4.7. Continues

83	CUPVSiirt1043	2	1.28	0.25	0.22	0.19	98-101
84	CUPVSiirt1047	3	2.91	0.75	0.66	0.58	127-155
85	CUPVSiirt1053	2	1.60	0.50	0.38	0.30	166-172
86	CUPVSiirt1055	2	2.00	0.33	0.50	0.38	151-163
87	CUPVSiirt1057	6	5.33	0.75	0.81	0.79	226-252
88	CUPVSiirt1062	3	2.91	0.75	0.66	0.58	144-159
89	CUPVSiirt1071	4	2.91	0.75	0.66	0.60	140-147
90	CUPVSiirt1116	6	5.33	1.00	0.81	0.79	158-188
91	CUPVSiirt1117	2	1.60	0.50	0.38	0.30	149-150
92	CUPVSiirt1120	2	1.28	0.25	0.22	0.19	197-208
93	CUPVSiirt1127	5	4.00	1.00	0.75	0.71	120-142
94	CUPVSiirt1145	2	1.28	0.25	0.22	0.19	162-168
95	CUPVSiirt1153	2	1.28	0.25	0.22	0.19	182-194
96	CUPVSiirt1171	6	5.33	0.75	0.81	0.79	229-249
97	CUPVSiirt1182	3	2.67	0.00	0.63	0.55	155-173
98	CUPVSiirt1183	2	1.60	0.00	0.38	0.30	240-243
99	CUPVSiirt1202	5	4.57	1.00	0.78	0.75	180-202
100	CUPVSiirt1224	3	2.67	0.00	0.63	0.55	265-275
101	CUPVSiirt1238	3	2.46	0.25	0.59	0.51	238-242
102	CUPVSiirt1243	5	4.57	0.25	0.78	0.75	134-164
103	CUPVSiirt1260	3	2.46	0.75	0.59	0.51	160-175
104	CUPVSiirt1271	2	1.28	0.25	0.22	0.19	231-234
105	CUPVSiirt1273	2	1.60	0.50	0.38	0.30	135-147
106	CUPVSiirt1278	3	2.13	0.25	0.53	0.47	177-194
107	CUPVSiirt1322	3	2.67	0.00	0.63	0.55	220-222
108	CUPVSiirt1326	7	6.40	1.00	0.84	0.82	187-208
109	CUPVSiirt1330	2	1.80	0.00	0.44	0.35	154-173
110	CUPVSiirt1331	2	1.28	0.25	0.22	0.19	97-98
111	CUPVSiirt1353	3	2.91	0.25	0.66	0.58	183-187

Table 4.7. Continues

112	CUPVSiirt1372	4	3.00	0.67	0.67	0.62	115-130
113	CUPVSiirt1378	6	5.33	0.75	0.81	0.79	87-119
114	CUPVSiirt1394	2	1.60	0.50	0.38	0.30	234-236
115	CUPVSiirt1399	2	1.28	0.25	0.22	0.19	204-210
116	CUPVSiirt1400	6	5.33	1.00	0.81	0.79	164-184
117	CUPVSiirt1402	2	1.28	0.25	0.22	0.19	178-181
118	CUPVSiirt1405	7	6.40	0.75	0.84	0.82	183-219
119	CUPVSiirt1406	7	6.40	0.75	0.84	0.82	188-208
120	CUPVSiirt1413	4	2.91	1.00	0.66	0.60	182-202
121	CUPVSiirt1417	3	3.00	0.00	0.67	0.59	134-150
122	CUPVSiirt1418	4	2.91	0.75	0.66	0.60	131-138
123	CUPVSiirt1431	2	1.28	0.25	0.22	0.19	198-200
124	CUPVSiirt1442	5	3.20	0.75	0.69	0.65	120-138
125	CUPVSiirt1477	3	2.13	0.75	0.53	0.47	116-118
126	CUPVSiirt1564	4	2.91	0.50	0.66	0.60	188-208
127	CUPVSiirt1567	4	3.56	0.25	0.72	0.67	189-205
128	CUPVSiirt1611	2	2.00	0.00	0.50	0.38	198-203
129	CUPVSiirt1626	3	2.67	0.00	0.63	0.55	130-134
130	CUPVSiirt1628	4	2.91	0.75	0.66	0.60	127-155
131	CUPVSiirt1652	3	2.67	0.50	0.63	0.55	159-176
132	CUPVSiirt1655	2	2.00	0.00	0.50	0.38	156-158
133	CUPVSiirt1658	4	2.91	0.75	0.66	0.60	199-209
134	CUPVSiirt1667	4	3.60	0.33	0.72	0.67	168-174
135	CUPVSiirt1688	3	2.46	0.50	0.59	0.51	160-174
136	CUPVSiirt1714	2	2.00	0.00	0.5	0.38	202-208
137	CUPVSiirt1742	4	4.00	0.00	0.75	0.70	167-203
138	CUPVSiirt1749	4	3.60	0.67	0.72	0.67	143-161
139	CUPVSiirt1759	4	3.56	1.00	0.72	0.67	137-146
140	CUPVSiirt1764	5	4.57	0.75	0.78	0.75	161-172
141	CUPVSiirt1768	4	2.91	1.00	0.66	0.60	108-123
142	CUPVSiirt1788	2	1.28	0.25	0.22	0.19	183-185
	Total	**485**					
	Mean	**3.40**	**2.8**	**0.47**	**0.56**	**0.50**	**73-266**

In *P. chinensis*, 177 (85.9%) loci amplified in SSR-PCR analysis, and 119 loci (67.2%) were polymorphic. A total of 365 alleles were amplified from 119 polymorphic SSR loci with an average of 3.1 alleles per locus. The highest number of allele (*Na* = 7) was in CUPVSiirt836 locus. The CUPVSiirt186, CUPVSiirt1330, CUPVSiirt1400 loci also had high number of alleles (*Na* = 6). The average observed heterozygosity (*Ho*) was 0.48 and it ranged between 0.00 and 1.00. The expected heterozygosity (*He*) values varied from 0.22 to 0.84 with an average of 0.54. The CUPVSiirt836 locus was more informative than the others due to high He value. The expected heterozygosity values of the CUPVSiirt1330, CUPVSiirt1400 loci were also high (0.81). The polymorphism information content (*PIC*) values ranged between 0.19 and 0.82 with an average of 0.48 in *P. chinensis.* The CUPVSiirt836 locus amplified the highest number of alleles and had the highest level of polymorphism. CUPVSiirt1330 and CUPVSiirt1400 loci had high *PIC* values in *P. chinensis* as well (Table 4.8).

Table 4.8. Number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), PIC values and allele range of 119 polymorphic SSR loci in *P. chinensis*.

No	Loci	*Na*	*Ne*	*Ho*	*He*	*PIC*	Allele range (bp)
1	CUPVSiirt15	3	1.68	0.50	0.41	0.37	104-108
2	CUPVSiirt22	2	1.88	0.25	0.47	0.36	180-181
3	CUPVSiirt26	2	2.00	0.50	0.50	0.38	170-173
4	CUPVSiirt37	2	1.28	0.25	0.22	0.19	155-167
5	CUPVSiirt71	3	2.67	0.00	0.63	0.55	128-134
6	CUPVSiirt95	2	1.28	0.25	0.22	0.19	196-216
7	CUPVSiirt121	3	1.68	0.50	0.41	0.37	1118-124
8	CUPVSiirt125	2	1.88	0.25	0.47	0.36	214-224

Table 4.8. Continued

9	CUPVSiirt129	4	2.91	0.75	0.66	0.60	140-169
10	CUPVSiirt149	3	2.67	0.50	0.63	0.55	98-102
11	CUPVSiirt151	2	1.88	0.25	0.47	0.36	158-172
12	CUPVSiirt171	2	2.00	0.00	0.50	0.38	138-142
13	CUPVSiirt186	6	4.57	0.75	0.78	0.75	160-167
14	CUPVSiirt213	4	3.20	1.00	0.69	0.63	211-223
15	CUPVSiirt230	3	2.13	0.75	0.53	0.47	177-187
16	CUPVSiirt238	3	3.00	0.00	0.67	0.59	171-191
17	CUPVSiirt242	3	2.46	1.00	0.59	0.51	129-138
18	CUPVSiirt243	2	1.28	0.25	0.22	0.19	137-138
19	CUPVSiirt256	3	2.57	0.33	0.61	0.54	173-183
20	CUPVSiirt271	3	2.67	0.75	0.63	0.55	98-118
21	CUPVSiirt284	4	2.91	0.50	0.66	0.60	229-251
22	CUPVSiirt297	3	2.91	0.25	0.66	0.58	141-144
23	CUPVSiirt308	2	2.00	0.00	0.50	0.38	169-170
24	CUPVSiirt312	5	4.57	0.50	0.78	0.75	154-172
25	CUPVSiirt333	5	3.20	1.00	0.69	0.65	142-150
26	CUPVSiirt349	3	2.57	1.00	0.61	0.54	164-170
27	CUPVSiirt357	2	1.60	0.50	0.38	0.30	181-185
28	CUPVSiirt436	4	3.20	0.75	0.69	0.63	91-101
29	CUPVSiirt472	5	4.00	1.00	0.75	0.71	181-203
30	CUPVSiirt476	2	2.00	0.00	0.50	0.38	145-149
31	CUPVSiirt496	4	3.60	0.67	0.72	0.67	203-239
32	CUPVSiirt501	2	2.00	1.00	0.50	0.38	148-158
33	CUPVSiirt505	3	2.13	0.25	0.53	0.47	154-170
34	CUPVSiirt509	2	1.60	0.50	0.38	0.30	168-175
35	CUPVSiirt543	3	2.67	0.50	0.63	0.55	111-125
36	CUPVSiirt565	5	4.57	0.50	0.78	0.75	147-174
37	CUPVSiirt568x	2	2.00	1.00	0.50	0.38	109-117

Table 4.8. Continued

38	CUPVSiirt625	3	2.67	0.00	0.63	0.55	183-191
39	CUPVSiirt649	3	2.91	1.00	0.66	0.58	165-177
40	CUPVSiirt660	3	2.00	0.33	0.50	0.45	131-142
41	CUPVSiirt712	2	1.80	0.00	0.44	0.35	204-210
42	CUPVSiirt719	2	1.60	0.50	0.38	0.3	209-212
43	CUPVSiirt724	2	1.28	0.25	0.22	0.19	1322-135
44	CUPVSiirt742	2	1.28	0.25	0.22	0.19	205-209
45	CUPVSiirt768	3	1.68	0.25	0.41	0.37	208-220
46	CUPVSiirt782	2	1.60	0.50	0.38	0.3	176-188
47	CUPVSiirt788	5	3.20	0.50	0.69	0.65	205-262
48	CUPVSiirt796	4	2.29	0.75	0.56	0.52	96-106
49	CUPVSiirt818	4	3.56	1.00	0.72	0.67	173-187
50	CUPVSiirt836	7	6.40	1.00	0.84	0.82	152-174
51	CUPVSiirt838	3	1.68	0.25	0.41	0.37	142-153
52	CUPVSiirt855	2	1.28	0.25	0.22	0.19	250-278
53	CUPVSiirt858	2	1.88	0.75	0.47	0.36	178-180
54	CUPVSiirt875	4	4.00	0.00	0.75	0.70	161-185
55	CUPVSiirt876	5	4.57	0.75	0.78	0.75	180-189
56	CUPVSiirt883	2	1.80	0.67	0.44	0.35	179-181
57	CUPVSiirt929	3	2.13	0.25	0.53	0.47	88-95
58	CUPVSiirt961	2	2.00	1.00	0.50	0.38	177-179
59	CUPVSiirt986	2	1.88	0.25	0.47	0.36	156-157
60	CUPVSiirt989	3	2.91	0.25	0.66	0.58	146-162
61	CUPVSiirt1003	2	1.88	0.75	0.47	0.36	73-96
62	CUPVSiirt1021	2	1.60	0.00	0.38	0.30	127-143
63	CUPVSiirt1047	2	1.38	0.33	0.28	0.24	125-131
64	CUPVSiirt1053	2	1.38	0.33	0.28	0.24	159-166
65	CUPVSiirt1062	4	2.29	0.75	0.56	0.52	144-172
66	CUPVSiirt1071	2	2.00	1.00	0.50	0.38	131-143

Table 4.8. Continued

67	CUPVSiirt1116	3	1.68	0.50	0.41	0.37	154-170
68	CUPVSiirt1117	2	2.00	0.00	0.50	0.38	148-149
69	CUPVSiirt1120	2	1.60	0.00	0.38	0.30	198-201
70	CUPVSiirt1122	2	2.00	1.00	0.50	0.38	121-210
71	CUPVSiirt1140	4	4.00	1.00	0.75	0.70	156-165
72	CUPVSiirt1145	3	2.67	0.75	0.63	0.55	154-166
73	CUPVSiirt1153	2	2.00	1.00	0.50	0.38	176-194
74	CUPVSiirt1171	5	4.00	0.75	0.75	0.71	229-261
75	CUPVSiirt1182	2	1.28	0.25	0.22	0.19	161-173
76	CUPVSiirt1202	5	4.00	1.00	0.75	0.71	190-212
77	CUPVSiirt1214	2	2.00	0.00	0.50	0.38	175-177
78	CUPVSiirt1224	4	2.91	0.75	0.66	0.60	265-292
79	CUPVSiirt1238	4	2.91	0.50	0.66	0.60	239-258
80	CUPVSiirt1243	2	2.00	0.00	0.50	0.38	140-142
81	CUPVSiirt1250	2	1.60	0.00	0.38	0.30	176-177
82	CUPVSiirt1260	4	2.91	0.75	0.66	0.60	154-184
83	CUPVSiirt1271	2	1.28	0.25	0.22	0.19	224-226
84	CUPVSiirt1273	2	1.28	0.25	0.22	0.19	139-140
85	CUPVSiirt1278	5	3.20	0.75	0.69	0.65	177-204
86	CUPVSiirt1326	3	2.13	0.25	0.53	0.47	186-212
87	CUPVSiirt1330	6	5.33	1.00	0.81	0.79	165-201
88	CUPVSiirt1345	4	3.60	0.67	0.72	0.67	156-191
89	CUPVSiirt1372	2	2.00	0.00	0.50	0.38	123-130
90	CUPVSiirt1378	3	2.13	0.50	0.53	0.47	84-99
91	CUPVSiirt1400	6	5.33	1.00	0.81	0.79	155-178
92	CUPVSiirt1402	2	1.60	0.50	0.38	0.30	172-181
93	CUPVSiirt1405	3	2.91	0.25	0.66	0.58	201-216
94	CUPVSiirt1406	3	1.68	0.50	0.41	0.37	188-198
95	CUPVSiirt1431	3	2.46	0.75	0.59	0.51	200-211

Table 4.8. Continued

96	CUPVSiirt1438	2	1.60	0.00	0.38	0.30	283-286
97	CUPVSiirt1442	4	4.00	0.00	0.75	0.70	114-124
98	CUPVSiirt1477	4	3.56	1.00	0.72	0.67	112-126
99	CUPVSiirt1478	3	1.68	0.50	0.41	0.37	92-96
100	CUPVSiirt1564	5	4.50	0.67	0.78	0.74	208-222
101	CUPVSiirt1567	4	4.00	0.00	0.75	0.70	181-193
102	CUPVSiirt1611	3	2.13	0.25	0.53	0.47	193-203
103	CUPVSiirt1626	3	2.67	0.00	0.63	0.55	125-130
104	CUPVSiirt1628	3	2.13	0.75	0.53	0.47	127-146
105	CUPVSiirt1639	2	1.38	0.33	0.28	0.24	168-170
106	CUPVSiirt1652	4	3.56	1.00	0.72	0.67	159-182
107	CUPVSiirt1655	3	1.68	0.50	0.41	0.37	158-163
108	CUPVSiirt1658	5	4.57	0.75	0.78	0.75	134-173
109	CUPVSiirt1667	3	2.00	0.67	0.50	0.45	164-170
110	CUPVSiirt1705	2	1.80	0.00	0.44	0.35	241-251
111	CUPVSiirt1714	2	1.60	0.50	0.38	0.30	202-208
112	CUPVSiirt1740	4	3.56	1.00	0.72	0.67	168-192
113	CUPVSiirt1742	3	2.67	0.00	0.63	0.55	161-191
114	CUPVSiirt1749	3	2.46	0.25	0.59	0.51	143-158
115	CUPVSiirt1759	3	2.91	0.25	0.66	0.58	141-146
116	CUPVSiirt1764	3	2.46	0.25	0.59	0.51	157-162
117	CUPVSiirt1768	4	3.60	1.00	0.72	0.67	108-119
118	CUPVSiirt1784	2	1.80	0.00	0.44	0.35	177-193
119	CUPVSiirt1788	2	2.00	0.33	0.50	0.38	183-185
	Total	**365**					
	Mean	**3.1**	**2.51**	**0.48**	**0.54**	**0.48**	**73-292**

In *P. lentiscus*, 151 (73.3%) SSR loci amplified with the lowest transferability rate among the five wild *Pistacia* species studied. Of the amplified SSR loci, 83 (55.0%) were polymorphic. A total of 217 alleles were obtained from 83 polymorphic SSR loci in *P. lentiscus*, ranging from 1 to 6, with an average of 2.6 alleles per locus. The highest number of alleles (*Na* = 6) was obtained from the CUPVSiirt1797 locus. The effective number of alleles ranged from 1.28 (CUPVSiirt446, CUPVSiirt476, CUPVSiirt565, CUPVSiirt838, CUPVSiirt847, CUPVSiirt1191, CUPVSiirt1224, CUPVSiirt1457 and CUPVSiirt1749) to 4.57 (CUPVSiirt1797) with an average of 2.13. The observed heterozigosity (*Ho*) values ranged from 0.00 to 1.00. The average observed heterozygosity (*Ho*) was 0.50, and the CUPVSiirt26, CUPVSiirt95, CUPVSiirt349, CUPVSiirt436, CUPVSiirt509, CUPVSiirt782, CUPVSiirt803, CUPVSiirt1021, CUPVSiirt1047, CUPVSiirt1053, CUPVSiirt1062, CUPVSiirt1567 and CUPVSiirt1797 loci were the most heterozygous. The expected heterozygosity (*He*) values varied from 0.22 to 0.78 with an average of 0.49. The CUPVSiirt1797 locus was more informative than the others due to its higher expected heterozygosity value, while CUPVSiirt446, CUPVSiirt476, CUPVSiirt565, CUPVSiirt838, CUPVSiirt847, CUPVSiirt1191, CUPVSiirt1224, CUPVSiirt1457 and CUPVSiirt1749 loci had the lowest values. The polymorphic information content (*PIC*) values ranged between 0.19 and 0.75 with an average of 0.41. The highest *PIC* value was obtained from CUPVSiirt1797 (0.75) and CUPVSiirt782 (0.70) loci. The highest values for *Na*, *Ne*, *He*, and *PIC* values were obtained from the CUPVSiirt1797 locus (Table 4.9).

Table 4.9. Number of alleles (Na), Number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), PIC values and allele range of 83 polymorphic SSR primer in *P. lentiscus*.

No	Loci	*Na*	*Ne*	*Ho*	*He*	*PIC*	Allele range (bp)
1	CUPVSiirt26	2	2.00	1.00	0.5	0.38	179-188
2	CUPVSiirt37	3	2.46	0.75	0.59	0.51	148-163
3	CUPVSiirt86	4	2.29	0.75	0.56	0.52	112-124
4	CUPVSiirt95	2	2.00	1.00	0.50	0.38	217-219
5	CUPVSiirt129	2	1.60	0.50	0.38	0.30	127-137
6	CUPVSiirt171	2	1.80	0.67	0.44	0.35	138-149
7	CUPVSiirt230	3	2.46	0.50	0.59	0.51	185-193
8	CUPVSiirt242	4	2.91	0.75	0.66	0.60	134-147
9	CUPVSiirt256	2	1.60	0.50	0.38	0.30	173-187
10	CUPVSiirt284	2	1.88	0.25	0.47	0.36	252-256
11	CUPVSiirt294	3	2.91	0.75	0.66	0.58	114-120
12	CUPVSiirt297	2	1.60	0.00	0.38	0.30	139-140
13	CUPVSiirt312	3	3.00	0.00	0.67	0.59	158-166
14	CUPVSiirt333	3	2.13	0.75	0.53	0.47	151-159
15	CUPVSiirt340	3	2.13	0.75	0.53	0.47	131-135
16	CUPVSiirt349	3	2.57	1.00	0.61	0.54	168-178
17	CUPVSiirt436	2	2.00	1.00	0.50	0.38	100-101
18	CUPVSiirt446	2	1.28	0.25	0.22	0.19	236-242
19	CUPVSiirt465	2	1.60	0.50	0.38	0.30	112-120
20	CUPVSiirt472	3	2.13	0.75	0.53	0.47	252-268
21	CUPVSiirt476	2	1.28	0.25	0.22	0.19	154-157
22	CUPVSiirt505	4	2.91	0.75	0.66	0.60	146-165
23	CUPVSiirt509	4	3.20	1.00	0.69	0.63	171-188
24	CUPVSiirt565	2	1.28	0.25	0.22	0.19	146-147

Table 4.9. Continued

25	CUPVSiirt569	3	2.57	0.33	0.61	0.54	95-102
26	CUPVSiirt660	3	2.67	0.75	0.63	0.55	138-146
27	CUPVSiirt719	2	1.88	0.25	0.47	0.36	190-198
28	CUPVSiirt742	3	2.13	0.50	0.53	0.47	218-232
29	CUPVSiirt743	3	2.00	0.33	0.50	0.45	159-173
30	CUPVSiirt768	3	2.57	0.67	0.61	0.54	206-212
31	CUPVSiirt782	4	4.00	1.00	0.75	0.70	180-202
32	CUPVSiirt794	3	1.68	0.50	0.41	0.37	206-208
33	CUPVSiirt796	2	1.60	0.50	0.38	0.30	106-112
34	CUPVSiirt803	2	2.00	1.00	0.50	0.38	240-256
35	CUPVSiirt836	4	3.56	0.25	0.72	0.67	144-168
36	CUPVSiirt838	2	1.28	0.25	0.22	0.19	139-142
37	CUPVSiirt847	2	1.28	0.25	0.22	0.19	249-253
38	CUPVSiirt876	4	2.91	0.50	0.66	0.60	187-203
39	CUPVSiirt956	3	2.67	0.00	0.63	0.55	131-135
40	CUPVSiirt961	2	1.80	0.00	0.44	0.35	190-194
41	CUPVSiirt986	3	2.46	0.75	0.59	0.51	146-153
42	CUPVSiirt1003	2	1.80	0.67	0.44	0.35	81-97
43	CUPVSiirt1008	2	1.80	0.00	0.44	0.35	163-165
44	CUPVSiirt1017	2	1.60	0.50	0.38	0.30	230-232
45	CUPVSiirt1021	3	2.46	1.00	0.59	0.51	127-135
46	CUPVSiirt1043	3	2.00	0.67	0.50	0.45	113-157
47	CUPVSiirt1047	2	2.00	1.00	0.50	0.38	133-144
48	CUPVSiirt1053	2	2.00	1.00	0.50	0.38	145-153
49	CUPVSiirt1055	2	1.80	0.67	0.44	0.35	175-180
50	CUPVSiirt1062	3	2.57	1.00	0.61	0.54	146-151
51	CUPVSiirt1071	2	1.38	0.33	0.28	0.24	126-135
52	CUPVSiirt1120	2	1.60	0.00	0.38	0.30	193-196
53	CUPVSiirt1145	4	3.60	0.67	0.72	0.67	150-168

Table 4.9. Continued

54	CUPVSiirt1153	2	1.88	0.75	0.47	0.36	172-194
55	CUPVSiirt1182	2	1.60	0.00	0.38	0.30	159-171
56	CUPVSiirt1191	2	1.28	0.25	0.22	0.19	154-164
57	CUPVSiirt1202	3	2.13	0.75	0.53	0.47	180-194
58	CUPVSiirt1224	2	1.28	0.25	0.22	0.19	268-273
59	CUPVSiirt1238	4	3.56	0.75	0.72	0.67	229-252
60	CUPVSiirt1243	2	1.60	0.00	0.38	0.30	134-136
61	CUPVSiirt1260	2	1.80	0.00	0.44	0.35	151-157
62	CUPVSiirt1278	2	1.60	0.00	0.38	0.30	217-220
63	CUPVSiirt1326	2	1.88	0.25	0.47	0.36	186-188
64	CUPVSiirt1345	3	2.57	0.33	0.61	0.54	173-191
65	CUPVSiirt1372	2	1.80	0.00	0.44	0.35	123-130
66	CUPVSiirt1400	3	2.91	0.75	0.66	0.58	169-197
67	CUPVSiirt1402	3	2.13	0.50	0.53	0.47	209-213
68	CUPVSiirt1405	4	3.56	0.25	0.72	0.67	191-223
69	CUPVSiirt1413	2	1.38	0.33	0.28	0.24	184-196
70	CUPVSiirt1438	2	1.38	0.33	0.28	0.24	266-286
71	CUPVSiirt1457	2	1.28	0.25	0.22	0.19	156-157
72	CUPVSiirt1477	2	1.88	0.25	0.47	0.36	118-120
73	CUPVSiirt1547	2	1.38	0.33	0.28	0.24	120-126
74	CUPVSiirt1567	2	2.00	1.00	0.50	0.38	189-203
75	CUPVSiirt1611	2	1.80	0.00	0.44	0.35	193-194
76	CUPVSiirt1652	2	1.60	0.00	0.38	0.30	167-172
77	CUPVSiirt1655	3	2.46	0.50	0.59	0.51	170-184
78	CUPVSiirt1667	2	1.60	0.50	0.38	0.30	149-152
79	CUPVSiirt1734	2	2.00	0.50	0.50	0.38	178-185
80	CUPVSiirt1749	2	1.28	0.25	0.22	0.19	133-143
81	CUPVSiirt1759	3	2.57	0.67	0.61	0.54	147-153
82	CUPVSiirt1764	4	3.20	0.50	0.69	0.63	155-176
83	CUPVSiirt1797	6	4.57	1.00	0.78	0.75	159-177
	Total	**217**					
	Mean	**2.6**	**2.13**	**0.50**	**0.49**	**0.41**	**81-286**

Polymorphic SSRs in all tested *Pistacia* species were found in this study. Of the analyzed 206 SSR loci, 41 were polymorphic, and 136 had amplifications in all tested six *Pistacia* species. Mean values for genetic parameters in each of the *Pistacia* species are shown in Table 4.10. The highest transferability was obtained in *P. atlantica* and *P. integerrima,* while *P. lentiscus* had the lowest transferability. In wild *Pistacia* species, the highest average number of alleles was obtained in *P. atlantica* and *P. terebinthus*, while *P. lentiscus* had the lowest number (Table 4.10).

Table 4.10. Averages of genetic diversity values for each of *Pistacia* species

Species	**Transferability (%)**	**Total number of polymorphic alleles**	***Na***	***Ne***	***Ho***	***He***	***PIC***
P. vera	206 (100%)	897	4.48	2.57	0.46	0.55	0.50
P. atlantica	200 (97.1%)	527	3.27	2.59	0.48	0.56	0.49
P. integerrima	193 (93.7%)	416	2.64	2.21	0.50	0.52	0.44
P. chinensis	177 (86.0%)	365	3.06	2.51	0.48	0.54	0.48
P. terebinthus	183 (88.9%)	485	3.41	2.80	0.47	0.56	0.50
P. lentiscus	151 (73.3%)	217	2.61	2.13	0.50	0.49	0.41
ALL	206 (100%)	2,036	9.88	4.67	0.41	0.74	0.71

From the 59,280 SSRs detected in the genome survey of pistachio in this study, primer pairs were designed for 950 loci. Initial screening of them for polymorphism and ease of scoring revealed that 206 SSR loci were selected for genetic diversity analysis of 44 *Pistacia* genotypes. In *P. vera*, 200 SSR loci were polymorphic and can be used in further studies such as genetic linkage mapping, germplasm characterization, fingerprinting, and genetic diversity.

Several reports have been published on SSR development in *P. vera* (Ahmad et al, 2003; Kolahi-Zonoozi et al, 2014; Zaloglu et al, 2015; Topcu et al, 2016; Khodaeiaminjan et al, 2017a). The average number of alleles per locus in *P. vera*

was 4.5 in this study and it was higher than Na values in pistachio in the literature who developed SSRs from *P. vera* by Topcu et al (2016; *Na* = 4.1), Ahmad et al (2003; *Na*=3.3), Zaloglu et al (2015; *Na* = 3.6) Kolahi-Zonoozi et al (2014; Na = 2.7) but lower than Na value (*Na*= 6.2) from Khodaeiaminjan et al (2017b). The average expected heterozygosity value in this study was higher than Arabnezhad et al (2011; *He* = 0.45), Topcu et al (2016; *He* = 0.52), Kolahi-Zonoozi et al (2014; *He* = 0.35) but lower than of SSRs developed by Khodaeiaminjan et al (2017b; *He* = 0.69) and Zaloglu et al (2015; *He* = 0.64).

Wild *Pistacia* species have been used commonly for rootstock seed sources and forest trees. Also 206 SSRs were tested loci in five wild *Pistacia* species for their possible use in genetic diversity studies. This study reports polymorphic SSR loci for each species: 161 for *P. atlantica*, 157 for *P. integerrima*, 142 for *P. terebinthus*, 119 for *P. chinensis*, and 83 polymorphic for *P. lentiscus*. Albaladejo et al (2008) developed only eight polymorphic SSR loci for *P. lentiscus*. Zaloglu et al (2015) and Topcu et al (2016) developed SSRs from *P. vera* and tested them for PCR amplification in wild *Pistacia* species. The authors did not analyze the SSR loci for polymorphism in the wild species. Therefore, this study describes an important number of novel polymorphic SSRs for each of five wild *Pistacia* species.

The transferability rates of SSRs in this study were high; this is consistent with findings from previous studies performed by Zaloglu et al (2015) and Topcu et al (2016). Taken together, these data demonstrate that SSRs are very powerful tools to be used in synteny analysis in *Pistacia*. The lowest transferability rate was obtained for *P. lentiscus*, which is one of the most distant species to *P. vera* in the genus (Kafkas et al, 2006a; Parfitt and Badenes, 1997).

4.2.5. Cluster Analysis and Genetic Structure in *Pistacia*

Cluster analysis was performed in 24 *P. vera* cultivars and 20 wild *Pistacia* genotypes using 136 SSR loci that amplified in all tested *Pistacia* species. UPGMA analysis showed that all *Pistacia* species and genotypes were clearly separated from each other. Two main clusters were observed: the first cluster contained all individuals from *P. vera*, whereas the second cluster included wild *Pistacia* species: *P. atlantica*, *P. integerrima*, *P. chinensis*, *P. terebinthus*, and *P. lentiscus* (Figure 4.6). *P. atlantica* was the closest species to *P. vera*, while *P. lentiscus* was the most distant.

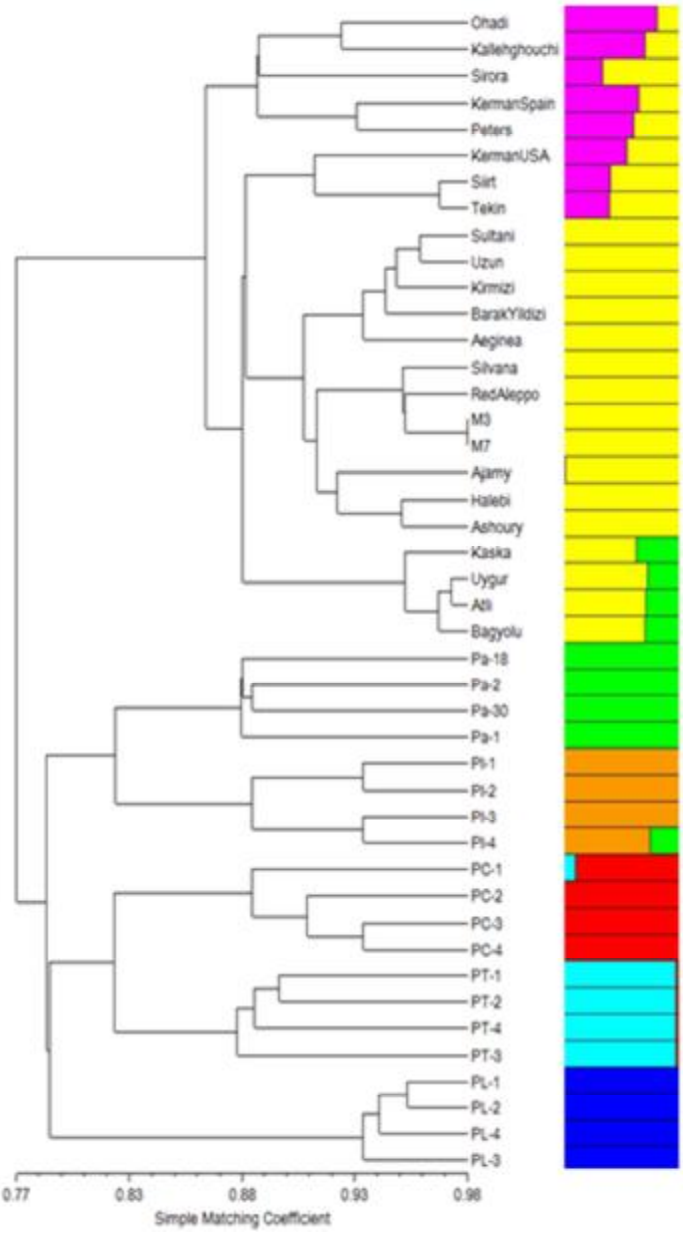


Figure 4.6. UPGMA dendrogram of 24 *P. vera* cultivars and 20 genotypes belongs to *P. atlantica*, *P. integerrima*, *P. terebinthus*, *P. chinensis* and *P. lentiscus.* The colors refers to population structure when K = 6, which all six *Pistacia* species were clearly separated.

The genetic structure of the *Pistacia* genotypes used in this study is shown in Figure 4.4. A model-based clustering method was performed for all 44 genotypes using 136 SSR loci. The most probable number of clusters were identified by calculating the Delta K (ΔK), which is based on the rate of change in the log probability of data between successive K values (K = 1 to K = 10). The peak of the ΔK graph corresponds to the most probable number of populations in the data set. The highest number of delta K (ΔK) was found at K = 2 (Figure 4.7), where all 44 genotypes were divided into two main groups similar to the UPGMA dendrogram (Figure 4.6). As the value for K increased to 3, the genotypes in group 2 were divided into two sub-groups: the first subgroup contained *P. lentiscus* and *P. terebinthus,* and the second subgroup contained the other wild *Pistacia* species. When K = 4, the wild *Pistacia* genotypes were divided into three subgroups: the first group included *P. lentiscus*, the second group contained *P. terebinthus* and *P. chinensis*, and the third group contained *P. atlantica* and *P. integerrima*. When K = 5, all *Pistacia* species were separated from each other, with the exception of *P. terebinthus* and *P. chinensis.* When K = 6, all six *Pistacia* species were clearly separated. When K = 7, the *Pistacia* species were again divided into six groups, and *P. vera* cultivars were grouped based on their origins, which was supported by another high ΔK found at K=7 (Figure 4.6, Figure 4.7, Figure 4.8). The cultivars originated from Iran were in one cluster, and the other cultivars were in the other cluster.

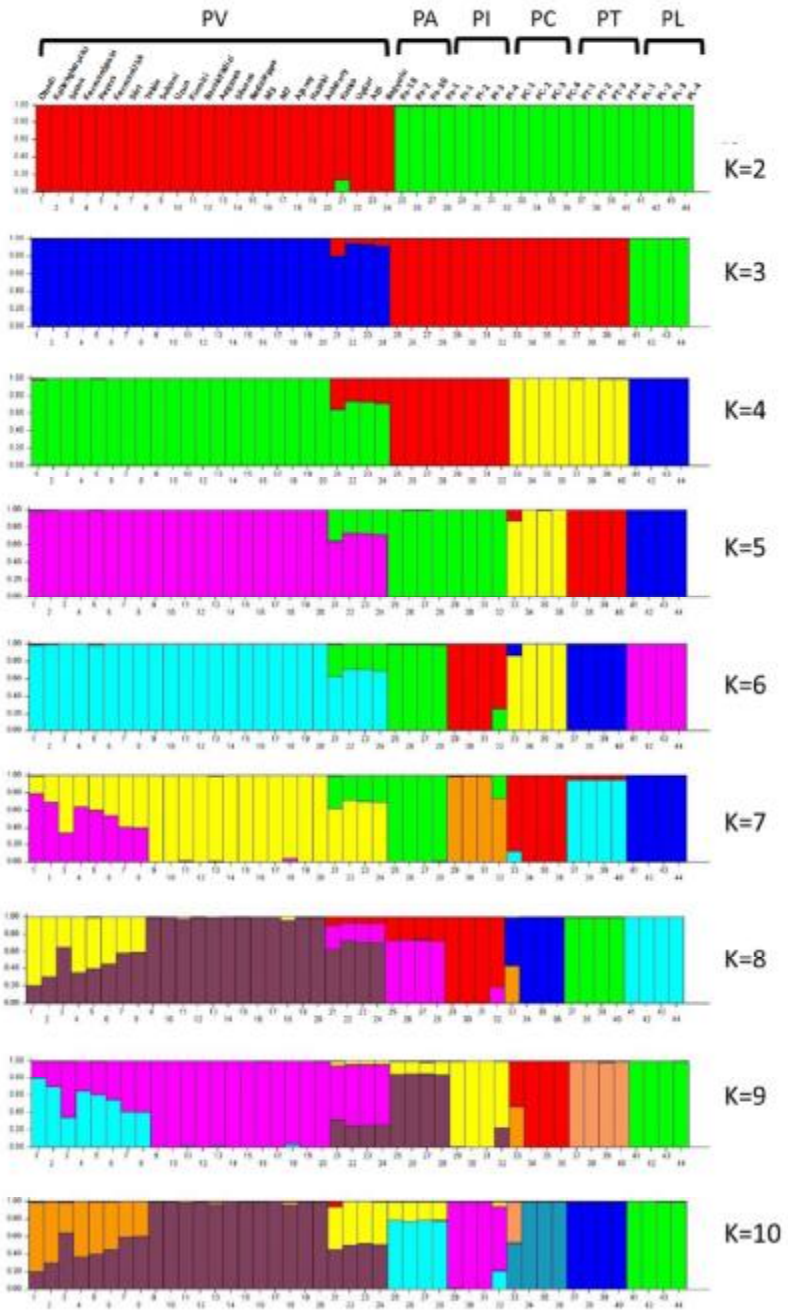


Figure 4.7. Population structure of 24 *P. vera* (PV) cultivars and 20 wild genotypes belong to *P. atlantica* (PA), *P. integerrima* (PI), *P. terebinthus* (PT), *P. chinensis* (PC) and *P. lentiscus* (PL). K = 2 to K = 10 represent the sub-populations.

All the genotypes and all six *Pistacia* species well-separated and consistently grouped in both cluster and structure analysis. The dendrogram and structure analysis at K = 2 divided *Pistacia* species into two main clusters: the first cluster included all *P. vera* cultivars, while the second cluster contained the wild genotypes belonging to five *Pistacia* species. The second cluster divided into two subclusters: the first subcluster contained *P. atlantica* and *P. integerrima* species, while the second subcluster contained *P. chinensis*, *P. terebinthus,* and *P. lentiscus* species. *P. atlantica* was the closest species to *P. vera*, whereas *P. lentiscus* was the most distant species. Similar results were obtained from other studies undertaking phylogenetic analysis in the genus *Pistacia* (Parfitt and Badenes, 1997; Kafkas and Perl-Treves, 2002; Kafkas, 2006a). Kafkas and Perl-Treves (2002a) divided the genus into two main groups: the first group included species with large, single-trunked trees, whereas the second group included species that mostly grow as shrubs or small trees. *P. terebinthus* and *P. lentiscus* were in the same group. These species were also clustered in the same group and were found to be the closest species in this study. A similar result was reported by Kafkas (2006a) using AFLP markers.

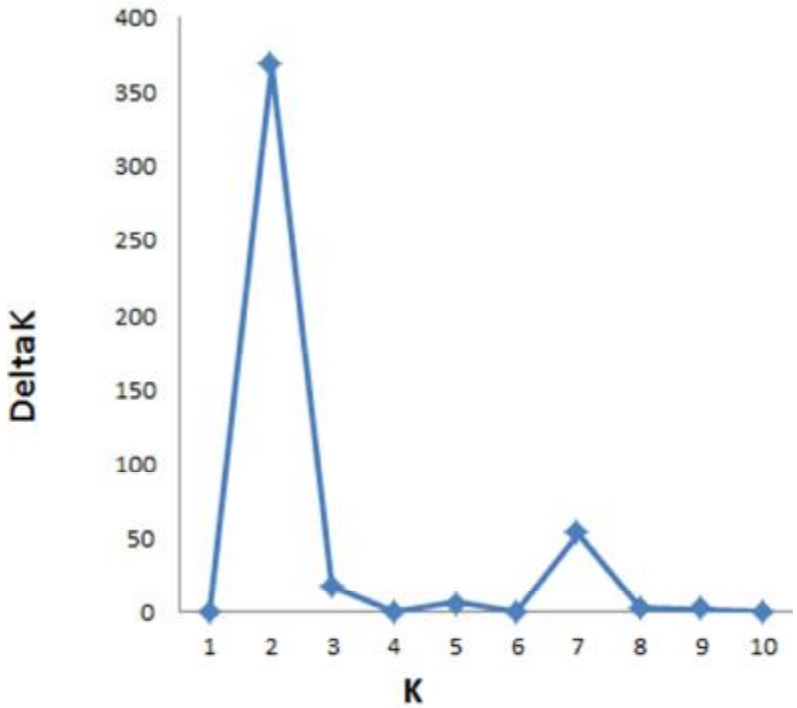


Figure 4.8. Values of DK. The modal value of this distribution is the true K(*) or the uppermost level of structure, here two (K = 2) clusters with 7 sub-clusters (K = 7).

The most comprehensive study on the relationships between *P. vera* cultivars was performed by Kafkas et al (2006a). The authors grouped 69 *P. vera* cultivars into two main groups: the first contained the cultivars originated from Iran, and the second included the cultivars from Mediterranean countries such as Turkey, Syria, and Greece. This study, also observed similar groupings and genetic relationships among 24 pistachio cultivars in this study. Structure analysis at K = 7 also supported the findings of the previous study performed by Kafkas et al (2006a), and was confirmed by the UPGMA clustering analysis in this study. Iranian cultivars such as Ohadi and Kalehghouchi were clustered in the Iranian group, while the other cultivars were clustered in the Mediterranean group.

The Australian cultivar, Sirora was in the Iranian group in this study, and this was supported by structure analysis at K=7. There were two Kerman clones in

the germplasm of the Pistachio Research Institute, which were introduced from Spain and USA. The clone introduced from Spain was clustered in the Iranian group and the other had a close relationship with the Siirt cultivar. Further study is necessary to elucidate the real Kerman in the germplasm. The male cultivars Atli, Uygur, Bağyolu, and Kaska were in the separate group within *P. vera* in the UPGMA dendrogram. The structure analysis demonstrated that these cultivars have a wild origin as they share an important number of alleles with the wild *Pistacia* species.

4.3. Genetic Linkage Map Construction by SSR Markers

In this study, three different genetic linkage maps were constructed: parental maps, consensus map and reference map. In the consensus map, all segregated SSRs were used, while only common markers were used in the construction of reference genetic linkage map. The parental maps included common markers, and SSR markers which are heterozygous only in one parent. The findings from each LG group are explained separately below. The data for reference map is given in a separate section.

4.3.1. LG1 of 'Siirt x Pa-18' F1 Population

P. vera cv. Siirt LG1 map was constructed by 22 markers with a total map length of 38.7 cM. The average distance between the markers was 1.8 cM and the average marker density was 0.57 markers per cM (supplementary file 1). The monoecious *P. atlantica* 'Pa-18' LG1 map had 17 markers and the total length was 23.4 cM. The average distance between SSR markers was 1.4 cM and the average marker density was 0.73 (supplementary file 2). The consensus LG1 map had 28 SSR markers with a total length of 41.6 cM (Figure 4.9 and Table 4.11).

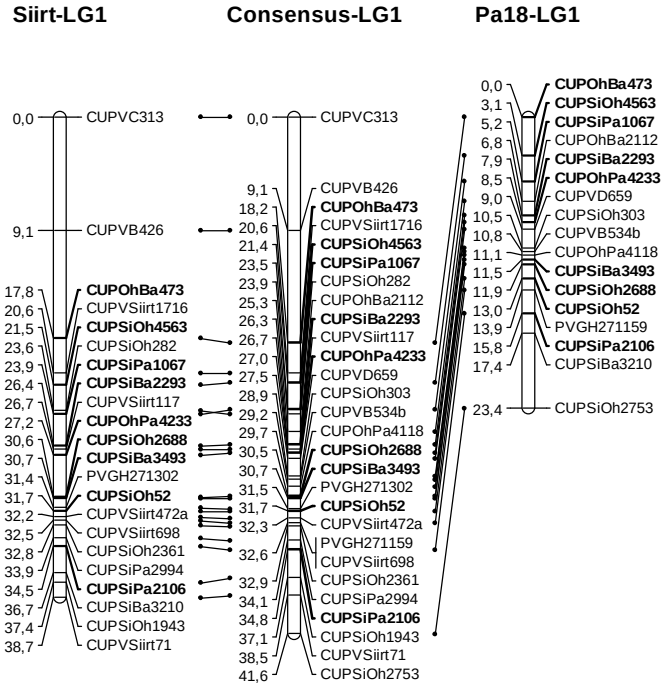


Figure 4.9. Parental and consensus maps of LG1 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

The abxcd, abxc0, efxeg, hkxhk, lmxll, lmx00, nnxnp and 00xnp segregations in the consensus map were 5, 2, 2, 0, 7, 5, 6 and 1, respectively. So, a total of 9 common markers were mapped in the consensus map of LG1. Of mapped 28 SSR markers, one marker (CUPSiOh2753) showed segregation distortion. The average distance between SSR markers was 1.5 cM and the average marker density was 0.67. The largest gap in LG1 consensus map was 9.1 cM between the markers CUPVC313 and CUPVB426 with CUPVB426 and CUPOhBa473 (Figure 4.9 and Table 4.11).

From 28 mapped markers in the consensus LG1 map, 5 markers were developed in this study and 17 markers from the study from Khodaeiamınjan et al (2017b) The rest of 6 mapped SSR markers were from studies performed by Zaloglu et al (2015), Topçu et al (2016) and Khodaeiaminjan et al (2017a; Figure 4.9 and Table 4.11).

Table 4.11. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG1 map of 'Siirt x Pa-18' F1 population.

No	Locus name	Position	Segregation type	X^2	Significance
1	CUPVC313	0.0	lmxll	0.07	-
2	CUPVB426	9.1	lmxll	0.03	-
3	CUPOhBa473	18.2	abxcd	4.18	-
4	CUPVSiirt1716	20.6	lmx00	0.40	-
5	CUPSiOh4563	21.4	abxc0	0.88	-
6	CUPSiPa1067	23.5	abxcd	4.73	-
7	CUPSiOh282	23.9	lmx00	0.79	-
8	CUPOhBa2112	25.3	nnxnp	0.20	-
9	CUPSiBa2293	26.3	efxeg	1.10	-
10	CUPVSiirt117	26.7	lmxll	0.10	-
11	CUPOhPa4233	27.0	abxcd	0.80	-
12	CUPVD659	27.5	nnxnp	0.39	-
13	CUPSiOh303	28.9	nnxnp	0.21	-
14	CUPVB534b	29.2	nnxnp	0.01	-
15	CUPOhPa4118	29.7	nnxnp	0.03	-
16	CUPSiOh2688	30.5	abxc0	0.19	-
17	CUPSiBa3493	30.7	abxcd	0.18	-
18	PVGH271302	31.5	lmxll	0.13	-
19	CUPSiOh52	31.7	efxeg	0.41	-
20	CUPVSiirt472a	32.3	lmx00	0.00	-
21	PVGH271159	32.6	nnxnp	0.07	-
22	CUPVSiirt698	32.6	lmxll	0.01	-
23	CUPSiOh2361	32.9	lmx00	0.03	-
24	CUPSiPa2994	34.1	lmxll	0.40	-
25	CUPSiPa2106	34.8	abxcd	0.35	-
26	CUPSiOh1943	37.1	lmxll	0.03	-
27	CUPVSiirt71	38.5	lmx00	0.04	-
28	CUPSiOh2753	41.6	00xnp	4.30	**

(**) $p<0.01$.

4.3.2. LG2 of 'Siirt x Pa-18' F1 Population

Nineteen markers with a total map length of 98.6 cM were maped in Siirt LG2 map. The average distance between the markers was 5.2 cM and the average

marker density was 0.19 markers per cM (supplementary file 1). LG2 of monoecious *P. atlantica* 'Pa-18' had 21 markers, and covered a total of 140.7 cM length. The average distance between the markers was 6.7 cM and the average marker density was 0.15 (supplementary file 2). Twenty-five SSR markers with a total length of 137.1 cM were linked in the consensus map of LG2. The numbers of abxcd, abxc0, efxeg, hkxhk, lmxll, lmx00 and nnxnp segregations in the consensus map were 8, 1, 2, 2, 3, 2 and 7, respectively. A total of 13 common markers were mapped in the LG2 consensus map of 'Siirt x Pa-18' F1 population. (Figure 4.10 and Table 4.12).

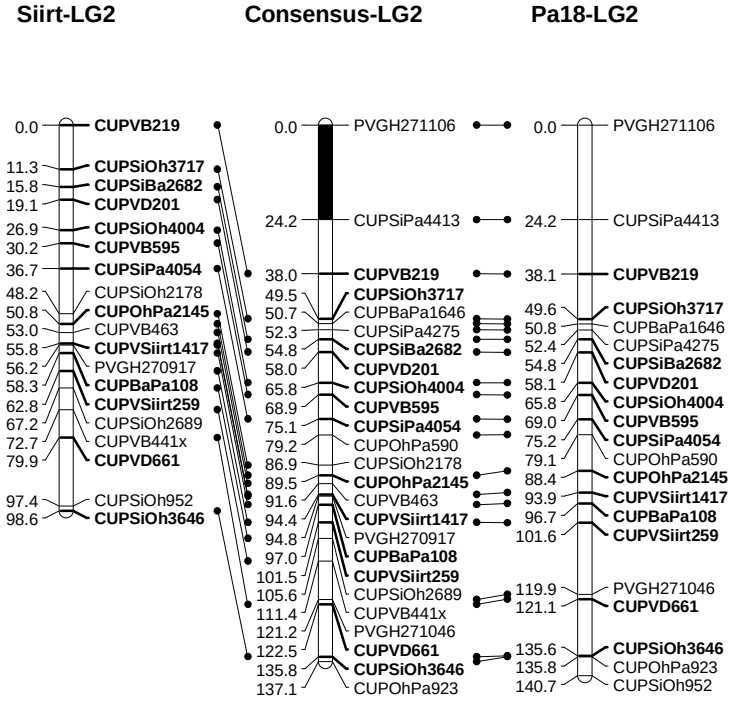


Figure 4.10. Parental and consensus maps of LG2 in 'Siirt x Pa-18' F1 population. The common markers are given in bold. The black segments represent the gaps ≥20 cM.

Among mapped 25 SSR markers in the consensus map, 11 SSR markers had distorted segregation. The average distance between SSR markers was 5.5 cM

and the average marker density was 0.18. The largest gap in LG2 consensus map was between PVGH271106 and CUPSiPa4413 markers with 24.2 cM. In this linkage group, 2 markers were mapped from this study and 14 SSR markers were Khodaeiaminjan et al (2017b) and the remaining 9 markers from other SSR development studies in the literature (Figure 4.10 and Table 4.12).

Table 4.12. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG2 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	PVGH271106	0.0	nnxnp	0.03	-
2	CUPSiPa4413	24.2	nnxnp	0.00	-
3	CUPVB219	38.0	abxcd	27.00	*******
4	CUPSiOh3717	49.5	abxcd	11.46	***
5	CUPBaPa1646	50.7	nnxnp	0.04	-
6	CUPSiPa4275	52.3	nnxnp	0.13	-
7	CUPSiBa2682	54.8	efxeg	5.72	-
8	CUPVD201	58.0	abxc0	9.65	**
9	CUPSiOh4004	65.8	abxcd	6.63	*
10	CUPVB595	68.9	abxcd	7.71	*
11	CUPSiPa4054	75.1	abxcd	1.74	-
12	CUPOhPa590	79.2	nnxnp	0.01	-
13	CUPSiOh2178	86.9	lmx00	3.90	**
14	CUPOhPa2145	89.5	abxcd	5.72	-
15	CUPVB463	91.6	lmxll	4.65	**
16	CUPVSiirt1417	94.4	hkxhk	8.34	**
17	PVGH270917	94.8	lmx00	3.23	*
18	CUPBaPa108	97.0	efxeg	4.62	-
19	CUPVSiirt259	101.5	abxcd	2.00	-
20	CUPSiOh2689	105.6	lmxll	4.88	**
21	CUPVB441x	111.4	lmxll	0.13	-
22	PVGH271046	121.2	nnxnp	2.89	*
23	CUPVD661	122.5	hkxhk	2.58	-
24	CUPSiOh3646	135.8	abxcd	4.13	-
25	CUPOhPa923	137.1	nnxnp	0.40	-

(*) $p<0.05$, (**) $p<0.01$, (***) $p<0.005$, (*******) $p<0.00005$.

4.3.3. LG3 of 'Siirt x Pa-18' F1 Population

'Siirt' LG3 linkage group contained 18 markers and spanned 71.5 cM. The average marker density was 0.25 markers per cM and the average distance between the markers was 4.0 cM (supplementary file 1). 'Pa-18' genotype LG3 map was constructed by 14 markers with a total linkage map length of 54.7 cM. The average distance between SSR markers was 3.9 cM and the average marker density was 0.26 (supplementary file 2). The linkage map for consensus LG3 map was constructed with 25 SSR markers and a total length of 76.9 cM. The numbers of abxcd, efxeg, lmxll, lmx00 and nnxnp segregations in the consensus map were 6, 1, 9, 2, and 7, respectively. So, a total of 7 common markers were mapped in the LG3 consensus map of 'Siirt x Pa-18' F1 population (Figure 4.11 and Table 4.13).

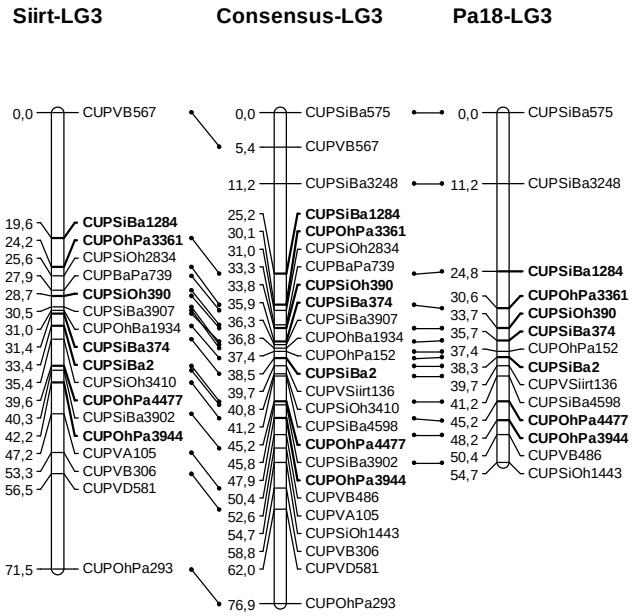


Figure 4.11. Parental and consensus maps of LG3 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

Five markers showed segregation distortion from 25 mapped SSR markers. The average distance between the markers was 3.1 cM and the average marker

density was 0.33. The largest gap in LG3 consensus map was 14 cM between the markers CUPSiBa3248 and CUPSiBa1284. From 25 mapped markers in the consensus LG3 map, one marker was developed in this study and 19 markers from the study by Khodaeiaminjan et al (2017b; Figure 4.11 and Table 4.13).

Table 4.13. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG3 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPSiBa575	0.0	nnxnp	9.80	****
2	CUPVB567	5.4	lmxll	1.14	-
3	CUPSiBa3248	11.2	nnxnp	2.89	*
4	CUPSiBa1284	25.2	abxcd	2.39	-
5	CUPOhPa3361	30.1	abxcd	3.40	-
6	CUPSiOh2834	31.0	lmx00	2.35	-
7	CUPBaPa739	33.3	lmxll	3.84	**
8	CUPSiOh390	33.8	abxcd	2.01	-
9	CUPSiBa374	35.9	abxcd	2.43	-
10	CUPSiBa3907	36.3	lmxll	1.83	-
11	CUPOhBa1934	36.8	lmxll	1.37	-
12	CUPOhPa152	37.4	nnxnp	0.22	-
13	CUPSiBa2	38.5	efxeg	0.98	-
14	CUPVSiirt136	39.7	nnxnp	0.01	-
15	CUPSiOh3410	40.8	lmx00	3.64	*
16	CUPSiBa4598	41.2	nnxnp	0.54	-
17	CUPOhPa4477	45.2	abxcd	2.20	-
18	CUPSiBa3902	45.8	lmxll	3.28	*
19	CUPOhPa3944	47.9	abxcd	1.26	-
20	CUPVB486	50.4	nnxnp	1.14	-
21	CUPVA105	52.6	lmxll	0.83	-
22	CUPSiOh1443	54.7	nnxnp	2.09	-
23	CUPVB306	58.8	lmxll	1.58	-
24	CUPVD581	62.0	lmxll	0.85	-
25	CUPOhPa293	76.9	lmxll	0.52	-

(*) $p<0.05$, (**) $p<0.01$, (****) $p<0.001$.

4.3.4. LG4 of 'Siirt x Pa-18' F1 Population

There were 21 markers in 'Siirt' LG4 map covering 124.8 cM with an average of 5.9 cM distance between the markers, and with 0.17 average marker density (supplementary file 1). Monoecious *P. atlantica* 'Pa-18' LG4 map had 19 markers, and the total LG length was 116.7 cM. The average distance between the markers was 6.1 cM and the average marker density was 0.16 (supplementary file 2). The consensus map of LG4 had 24 SSR markers with a total length of 124 cM. There were 8 'abxcd', 2 'abxc0', 1 'a0xcd' 5 'efxeg', 5 'lmxll', 2 'nnxnp' and 1 '00xnp'segregated markers in the consensus map. A total of 16 common markers were mapped in LG4 (Figure 4.12 and Table 4.14).

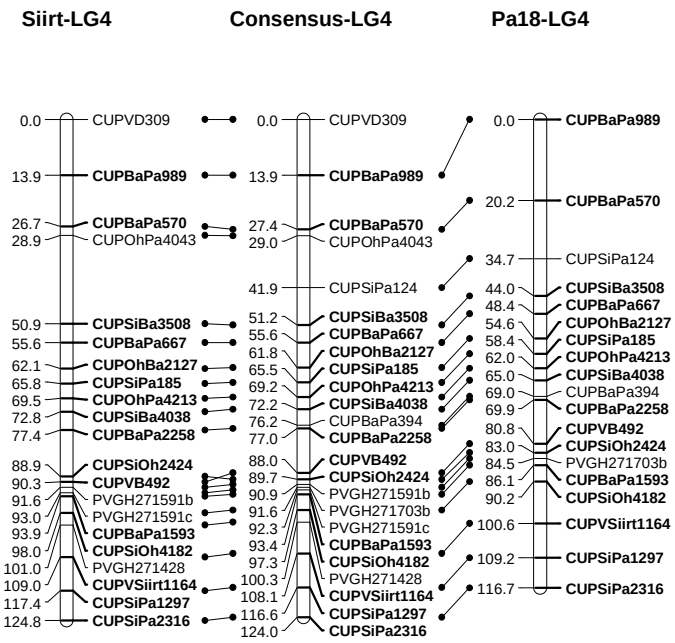


Figure 4.12. Parental and consensus maps of LG4 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

Of 24 mapped SSR markers in the consensus map, 5 SSR markers had distorted segregation and two of them had the high degree of segregation distortion. The average distance between SSR markers was 5.2 cM and the average marker

density was 0.19. There were two large gaps in LG4 between CUPVD309 and CUPBaPa989, and between CUPOhPa4043 and CUPSiPa124 markers with 13.9 cM and 11.9 cM length, respectively. In the consensus LG4 map, from 24 mapped markers, one mapped marker was developed in this study and 17 markers from Khodaeiaminjan et al (2017b; Figure 4.12 and Table 4.14).

Table 4.14. Locus names, positions (cM), segregation types, chi-square values (X2) and significant levels of distorted SSR loci in the consensus LG4 map of 'Siirt x Pa-18' F1 population

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPVD309	0.0	lmxll	23.14	*******
2	CUPBaPa989	13.9	efxeg	33.70	*******
3	CUPBaPa570	27.4	abxcd	6.93	*
4	CUPOhPa4043	29.0	lmxll	1.37	-
5	CUPSiPa124	41.9	nnxnp	0.13	-
6	CUPSiBa3508	51.2	abxcd	2.97	-
7	CUPBaPa667	55.6	abxcd	7.97	**
8	CUPOhBa2127	61.8	abxcd	1.68	-
9	CUPSiPa185	65.5	efxeg	7.29	*
10	CUPOhPa4213	69.2	a0xcd	5.45	-
11	CUPSiBa4038	72.2	efxeg	2.20	-
12	CUPBaPa394	76.2	00xnp	0.08	-
13	CUPBaPa2258	77.0	efxeg	1.56	-
14	CUPVB492	88.0	abxc0	2.65	-
15	CUPSiOh2424	89.7	abxc0	2.30	-
16	PVGH271591b	90.9	lmxll	0.81	-
17	PVGH271703b	91.6	nnxnp	0.07	-
18	PVGH271591c	92.3	lmxll	1.00	-
19	CUPBaPa1593	93.4	abxcd	1.37	-
20	CUPSiOh4182	97.3	efxeg	0.88	-
21	PVGH271428	100.3	lmxll	0.13	-
22	CUPVSiirt1164	108.1	abxcd	0.56	-
23	CUPSiPa1297	116.6	abxcd	2.18	-
24	CUPSiPa2316	124.0	abxcd	1.43	-

(*) $p<0.05$, (**) $p<0.01$, (*******) $p<0.00005$.

4.3.5. LG5 of 'Siirt x Pa-18' F1 Population

The length of 'Siirt' LG5 map was 103.2 cM and included 28 SSR markers. The average distance between the markers was 3.7 cM and the average marker density was 0.27 markers per cM (supplementary file 1). 'Pa-18' LG5 map had 22 markers with a total length of 99.7 cM. The average distance between SSR markers was 4.5 cM and the average marker density was 0.22 (supplementary file 2). The consensus LG5 map had 30 SSR markers with a total length of 102.8 cM. The numbers of abxcd, abxc0, a0xcd, efxeg, hkxhk, lmxll, lmx00 and nnxnp segregations in the consensus map were 14, 2, 1, 2, 1, 6, 2 and 2 respectively. So, a total of 20 common markers were mapped in the LG5 consensus map. Among mapped markers in LG5 consensus map. From 30 mapped markers in the consensus LG5, 6 markers were from this study and 19 markers were from Khodaeiamınjan et al (2017b; Figure 4.13 and Table 4.15).

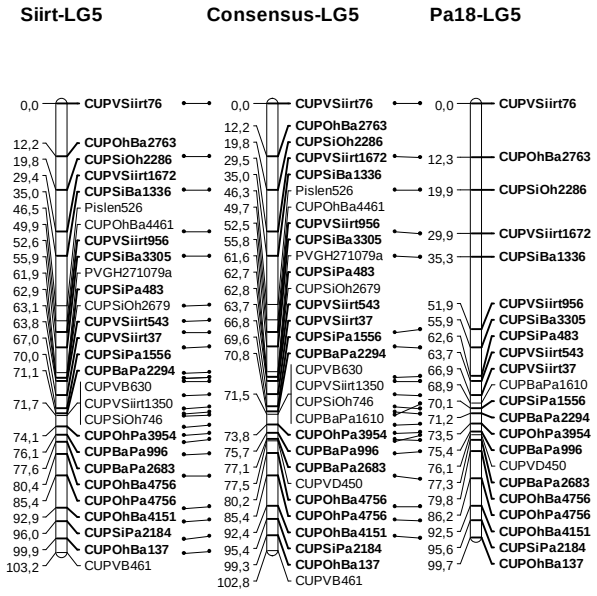


Figure 4.13. Parental and consensus maps of LG5 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

Of mapped 30 SSR markers, 13 markers showed segregation distortion. The average marker density was 0.29 cM and the average distance between SSR markers was 3.4 cM. The largest gap in LG5 consensus map was 12.2 cM between the markers CUPVSiirt76 and CUPOhBa2763 (Figure 4.12 and Table 4.15).

Table 4.15. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG5 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPVSiirt76	0.0	a0xcd	25.15	*******
2	CUPOhBa2763	12.2	abxcd	4.32	-
3	CUPSiOh2286	19.8	abxcd	3.94	-
4	CUPVSiirt1672	29.5	abxcd	0.33	-
5	CUPSiBa1336	35.0	abxcd	1.88	-
6	Pislen526	46.3	lmx00	0.03	-
7	CUPOhBa4461	49.7	lmxll	0.52	-
8	CUPVSiirt956	52.5	abxc0	2.65	-
9	CUPSiBa3305	55.8	abxcd	9.02	**
10	PVGH271079a	61.6	lmxll	2.10	-
11	CUPSiPa483	62.7	hkxhk	3.03	-
12	CUPSiOh2679	62.8	lmx00	3.23	*
13	CUPVSiirt543	63.7	efxeg	3.86	-
14	CUPVSiirt37	66.8	abxcd	3.53	-
15	CUPSiPa1556	69.6	abxcd	8.88	**
16	CUPBaPa2294	70.8	abxcd	5.94	-
17	CUPVB630	71.5	lmxll	2.31	-
18	CUPVSiirt1350	71.5	lmxll	2.89	*
19	CUPSiOh746	71.5	lmxll	2.61	-
20	CUPBaPa1610	71.5	nnxnp	2.31	-
21	CUPOhPa3954	73.8	efxeg	13.23	****
22	CUPBaPa996	75.7	abxcd	10.50	**
23	CUPBaPa2683	77.1	abxcd	9.56	**
24	CUPVD450	77.5	nnxnp	0.97	-
25	CUPOhBa4756	80.2	abxcd	4.50	-
26	CUPOhPa4756	85.4	abxcd	14.52	****
27	CUPOhBa4151	92.4	abxcd	7.14	*
28	CUPSiPa2184	95.4	abxcd	8.19	**
29	CUPOhBa137	99.3	abxcd	13.29	****
30	CUPVB461	102.8	lmxll	8.21	****

(*) $p<0.05$, (**) $p<0.01$, (****) $p<0.001$, (*******) $p<0.00005$.

4.3.6. LG6 in of 'Siirt x Pa-18' F1 Population

The 'Siirt' LG6 map was constructed by 16 markers with a total map length of 123.2 cM. The average distance between the markers was 7.7 cM and the average marker density was 0.13 markers per cM (supplementary file 1). 'Pa-18' LG6 had 20 markers, and the total LG length was 123.2 cM. The average distance between the markers was 6.2 cM and the average marker density was 0.16 (supplementary file 2).

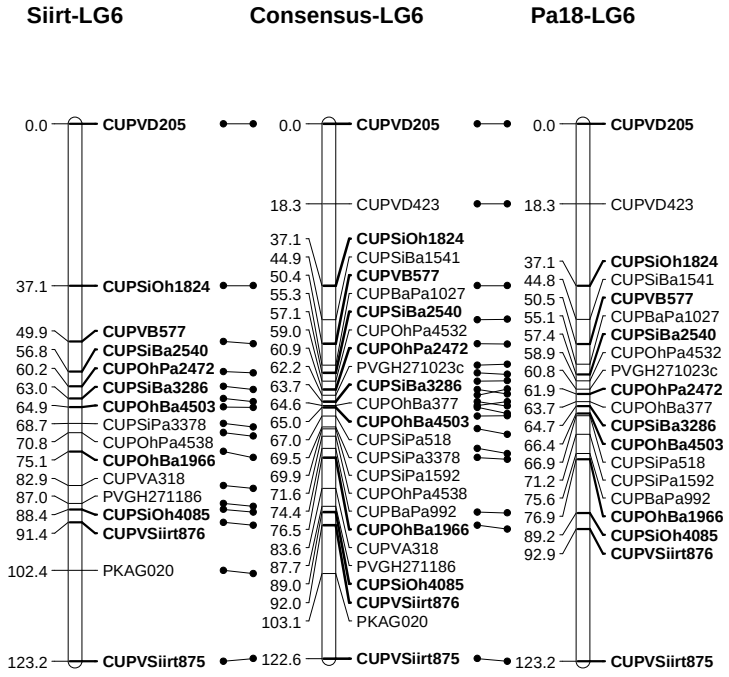


Figure 4.14. Parental and consensus maps of LG6 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

The consensus map of LG6 had 25 SSR markers with a total length of 122.6 cM. There were 6 'abxcd', 2 'abxc0', 1 'a0xcd', 2 'efxeg', 4 'lmxll', 1 'lmx00' and 9 'nnxnp' segregated markers in the LG6. A total of 11 common markers were mapped in the consensus LG6 map of 'Siirt x Pa-18' F1 population. Six markers showed segregation distortion in this linkage group. The average

distance between SSR markers was 4.9 cM and the average marker density was 0.20. The largest gap in consensus LG6 map was between CUPD205 and CUPD423 markers with 10.5 cM (Figure 4.14 and Table 4.16). Of the 25 markers in the consensus LG6 map, 2 markers were developed in this study, and 16 markers were from Khodaeiaminjan et al (2017b; Figure 4.14 and Table 4.16).

Table 4.16. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG6 map of 'Siirt x Pa-18' F1 population

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPVD205	0.0	efxeg	27.26	*******
2	CUPVD423	18.3	nnxnp	10.29	****
3	CUPSiOh1824	37.1	abxc0	5.21	-
4	CUPSiBa1541	44.9	nnxnp	1.35	-
5	CUPVB577	50.4	abxcd	2.63	-
6	CUPBaPa1027	55.3	nnxnp	2.17	-
7	CUPSiBa2540	57.1	abxcd	4.32	-
8	CUPOhPa4532	59.0	nnxnp	2.43	-
9	CUPOhPa2472	60.9	efxeg	4.31	-
10	PVGH271023c	62.2	nnxnp	0.65	-
11	CUPSiBa3286	63.7	abxcd	2.61	-
12	CUPOhBa377	64.6	nnxnp	2.61	-
13	CUPOhBa4503	65.0	abxcd	1.94	-
14	CUPSiPa518	67.0	nnxnp	0.53	-
15	CUPSiPa3378	69.5	lmxll	3.53	*
16	CUPSiPa1592	69.9	nnxnp	7.50	***
17	CUPOhPa4538	71.6	lmx00	1.80	-
18	CUPBaPa992	74.4	nnxnp	1.96	-
19	CUPOhBa1966	76.5	abxc0	7.40	*
20	CUPVA318	83.6	lmxll	4.30	**
21	PVGH271186	87.7	lmxll	6.22	**
22	CUPSiOh4085	89.0	abxcd	11.80	***
23	CUPVSiirt876	92.0	abxcd	11.05	**
24	PKAG020	103.1	lmxll	2.06	-
25	CUPVSiirt875	122.6	a0xcd	82.81	*******

(*) $p<0.05$, (**) $p<0.01$, (***) $p<0.005$, (****) $p<0.001$, (*******) $p<0.00005$.

4.3.7. LG7 of 'Siirt x Pa-18' F1 Population

Seventeen markers were linked in 'Siirt' LG7 map with a total map length of 80.9 cM. 'Pa-18' LG7 map had 21 markers, and the total length was 88.9 cM. In 'Siirt' LG7 map, the average distance between the markers was 4.8 cM and the average marker density was 0.21 markers per cM (supplementary file 1) and the average distance between SSR markers in 'Pa-18' LG7 map was 4.2 cM and the average marker density was 0.24 (supplementary file 2).

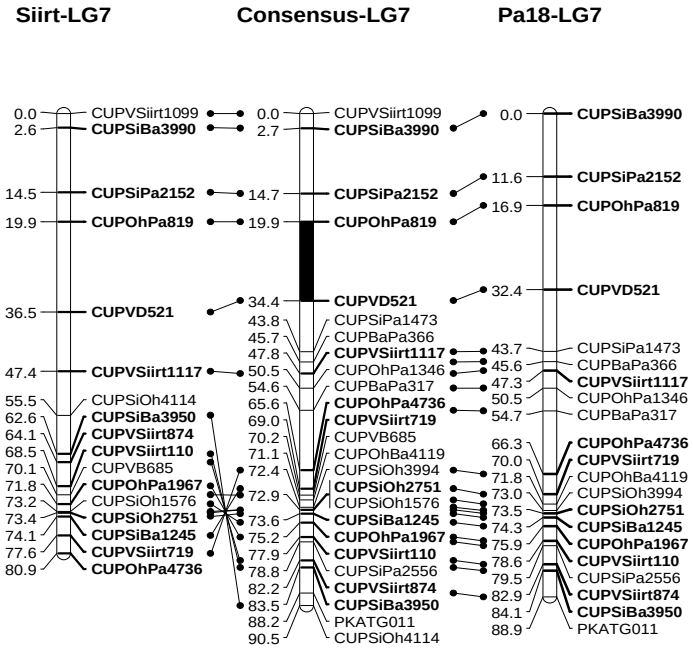


Figure 4.15. Parental and consensus maps of LG7 in 'Siirt x Pa-18' F1 population. The common markers are given in bold. The black segments represent the gaps ≥20 cM.

The consensus map of LG7 had 25 SSR markers with a total length of 90.5 cM. The numbers of abxcd, abxc0, efxeg, hkxhk, lmxll, lmx00, and nnxnp segregations in the consensus map were 7, 3, 1, 2, 1, 3 and 8 respectively. So, a total of 13 common markers were mapped in the LG7 consensus map. Of mapped 25 SSR markers, three markers had segregation distortion. The average distance

between SSR markers was 3.6 cM and the average marker density was 0.28. The largest gap in LG7 consensus map was 14.5 cM between the markers CUPOhPa819 and CUPVD521. Five markers from 25 mapped markers were developed in this study and 17 were from a SSR development study performed by Khodaeiaminjan et al (2017b). The rest of 3 mapped markers were developed from other studies in the literature (Figure 4.15 and Table 4.17).

Table 4.17. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG7 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPVSiirt1099	0.0	lmx00	1.16	-
2	CUPSiBa3990	2.7	abxcd	5.05	-
3	CUPSiPa2152	14.7	hkxhk	0.05	-
4	CUPOhPa819	19.9	hkxhk	1.96	-
5	CUPVD521	34.4	abxc0	11.66	***
6	CUPSiPa1473	43.8	nnxnp	0.97	-
7	CUPBaPa366	45.7	nnxnp	0.65	-
8	CUPVSiirt1117	47.8	abxcd	6.27	*
9	CUPOhPa1346	50.5	nnxnp	0.40	-
10	CUPBaPa317	54.6	nnxnp	1.14	-
11	CUPOhPa4736	65.6	efxeg	3.60	-
12	CUPVSiirt719	69.0	abxc0	0.54	-
13	CUPVB685	70.2	lmxll	1.83	-
14	CUPOhBa4119	71.1	nnxnp	0.13	-
15	CUPSiOh3994	72.4	nnxnp	0.83	-
16	CUPSiOh2751	72.9	abxcd	4.32	-
17	CUPSiOh1576	72.9	lmx00	2.61	-
18	CUPSiBa1245	73.6	abxc0	4.71	-
19	CUPOhPa1967	75.2	abxcd	7.14	*
20	CUPVSiirt110	77.9	abxcd	2.07	-
21	CUPSiPa2556	78.8	nnxnp	0.30	-
22	CUPVSiirt874	82.2	abxcd	1.56	-
23	CUPSiBa3950	83.5	abxcd	3.29	-
24	PKATG011	88.2	nnxnp	0.30	-
25	CUPSiOh4114	90.5	lmx00	1.57	-

(*) $p<0.05$, (***) $p<0.005$.

4.3.8. LG8 of 'Siirt x Pa-18' F1 Population

'Siirt' LG8 map was constructed by 16 markers with a total map length of 77.9 cM. The average distance between the markers was 4.9 cM and the average marker density was 0.21 markers per cM (supplementary file 1). 'Pa-18' LG8 map had 16 markers, and the total LG length was 79.0 cM. The average distance between the markers was 4.9 cM and the average marker density was 0.20 (supplementary file 2). The consensus LG8 map had 19 SSR markers with a total length of 76.4 cM. The numbers of abxcd, abxc0, efxeg, lmx00 and nnxnp segregations in the consensus map were 7, 2, 4, 3, and 3 respectively. A total of 13 common markers were mapped in the LG8 consensus map of 'Siirt x Pa-18' F1 population (Figure 4.16 and Table 4.18).

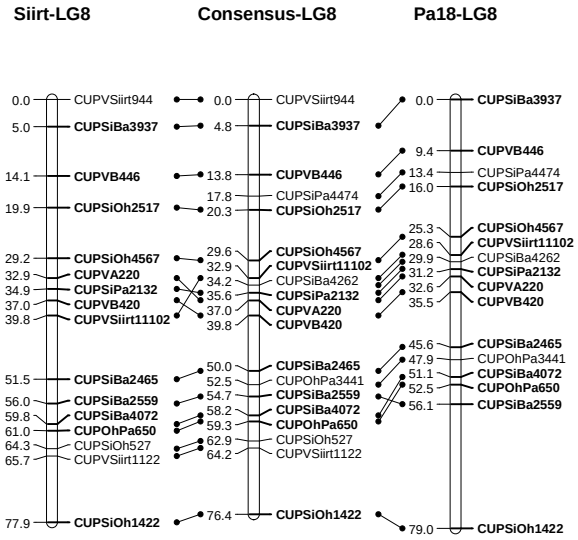


Figure 4.16. Parental and consensus maps of LG8 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

Among mapped 19 SSR markers in the LG8 consensus map, 8 SSRs were distorted markers. The average distance between SSR markers was 4.0 cM and the average marker density was 0.25. There was a large gap at the bottom of LG8 with

12.2 cM length between the CUPSiirt1122 and CUPSiOh1422 markers. Of mapped markers, three were from this study and 13 were from a study performed by Khodaeaminjan et al (2017b; Figure 4.16 and Table 4.18).

Table 4.18. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG8 map of 'Siirt x Pa-18' F1 population

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPVSiirt944	0.0	lmx00	3.64	*
2	CUPSiBa3937	4.8	abxcd	2.75	-
3	CUPVB446	13.8	efxeg	7.94	**
4	CUPSiPa4474	17.8	nnxnp	0.00	-
5	CUPSiOh2517	20.3	abxcd	2.26	-
6	CUPSiOh4567	29.6	efxeg	2.01	-
7	CUPVSiirt11102	32.9	efxeg	4.06	-
8	CUPSiBa4262	34.2	nnxnp	1.14	-
9	CUPSiPa2132	35.6	abxcd	6.26	*
10	CUPVA220	37.0	abxc0	6.70	*
11	CUPVB420	39.8	abxcd	7.87	**
12	CUPSiBa2465	50.0	abxcd	9.03	**
13	CUPOhPa3441	52.5	nnxnp	0.82	-
14	CUPSiBa2559	54.7	efxeg	4.26	-
15	CUPSiBa4072	58.2	abxcd	3.86	-
16	CUPOhPa650	59.3	abxcd	5.27	-
17	CUPSiOh527	62.9	lmx00	2.93	*
18	CUPVSiirt1122	64.2	lmx00	0.42	-
19	CUPSiOh1422	76.4	abxc0	13.74	****

(*) $p<0.05$, (**) $p<0.01$, (****) $p<0.001$.

4.3.9. LG9 of 'Siirt x Pa-18' F1 Population

'Siirt' LG9 map was 129.4 cM length having 32 SSR markers. The average distance between the markers was 4.0 cM and the average marker density was 0.25 markers per cM (supplementary file 1). Twenty-eight markers were mapped in LG9 of 'Pa-18' genotype had a total length of 131.9 cM. The average distance between SSR markers was 4.7 cM and the average marker density was 0.21 (supplementary file 2). The consensus map of LG9 had 32 SSR markers with a total length of 129.4 cM. The numbers of abxcd, a0xcd, efxeg, lmxll and lmx00 segregations in the consensus map were 22, 1, 5, 3 and 1, respectively. Consequently, a total of 28 common markers were mapped in the LG9 consensus map. Among mapped 32 markers, 8 markers were developed from present study and the remaining 24 SSR markers were from Khodaeiaminjan et al (2017b; Figure 4.17 and Table 4.19).

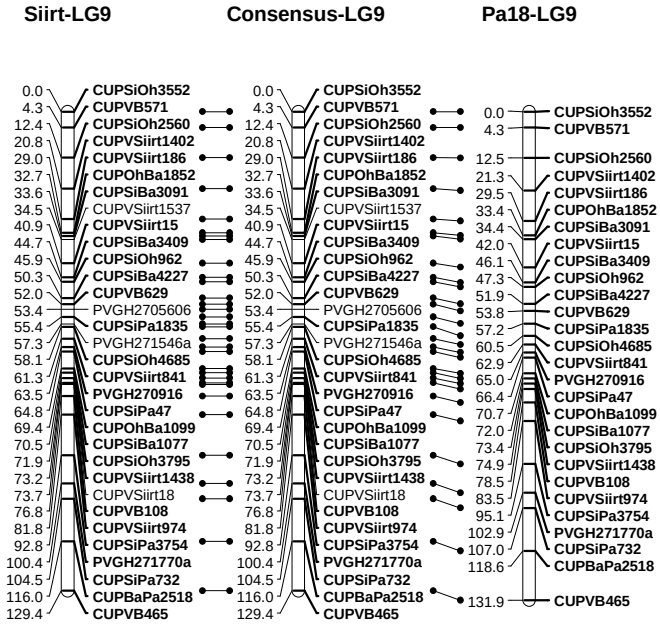


Figure 4.17. Parental and consensus maps of LG9 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

Table 4.19. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG9 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPSiOh3552	0.0	abxcd	2.72	-
2	CUPVB571	4.3	abxcd	2.20	-
3	CUPSiOh2560	12.4	abxcd	7.18	*
4	CUPVSiirt1402	20.8	abxcd	6.04	-
5	CUPVSiirt186	29.0	abxcd	3.77	-
6	CUPOhBa1852	32.7	abxcd	3.03	-
7	CUPSiBa3091	33.6	abxcd	4.63	-
8	CUPVSiirt1537	34.5	lmxll	2.31	-
9	CUPVSiirt15	40.9	abxcd	2.34	-
10	CUPSiBa3409	44.7	efxeg	5.17	-
11	CUPSiOh962	45.9	efxeg	5.08	-
12	CUPSiBa4227	50.3	abxcd	6.95	*
13	CUPVB629	52.0	abxcd	6.53	*
14	PVGH2705606	53.4	lmxll	0.52	-
15	CUPSiPa1835	55.4	abxcd	3.71	-
16	PVGH271546a	57.3	lmxll	0.20	-
17	CUPSiOh4685	58.1	abxcd	5.42	-
18	CUPVSiirt841	61.3	abxcd	0.30	-
19	PVGH270916	63.5	efxeg	1.30	-
20	CUPSiPa47	64.8	abxcd	0.22	-
21	CUPOhBa1099	69.4	abxcd	1.08	-
22	CUPSiBa1077	70.5	abxcd	0.95	-
23	CUPSiOh3795	71.9	abxcd	0.54	-
24	CUPVSiirt1438	73.2	abxcd	0.88	-
25	CUPVSiirt18	73.7	lmx00	0.13	-
26	CUPVB108	76.8	abxcd	2.93	-
27	CUPVSiirt974	81.8	a0xcd	3.35	-
28	CUPSiPa3754	92.8	abxcd	3.87	-
29	PVGH271770a	100.4	efxeg	8.02	**
30	CUPSiPa732	104.5	efxeg	7.32	*
31	CUPBaPa2518	116.0	abxcd	16.09	****
32	CUPVB465	129.4	abxcd	7.38	*

(*) $p<0.05$, (**) $p<0.01$, (****) $p<0.001$

Of mapped 32 SSR markers in LG9, seven SSRs were distorted markers. The average distance between SSR markers was 4.0 cM and the average marker density was 0.25. The largest gap in LG9 consensus map was 13.4 cM between the CUPBaPa2518 and CUPVB465 markers (Figure 4.17 and Table 4.19).

4.3.10. LG10 of 'Siirt x Pa-18' F1 Population

A total of 22 markers were mapped in LG10 of 'Siirt' cultivar with 87.8 cM length. The average distance between the markers was 4.0 cM and the average marker density was 0.25 markers per cM (supplementary file 1). LG10 of 'Pa-18' genotype had 18 markers, and the total LG length was 98.4 cM. The average distance between the markers was 5.5 cM and the average marker density was 0.18 (supplementary file 2).

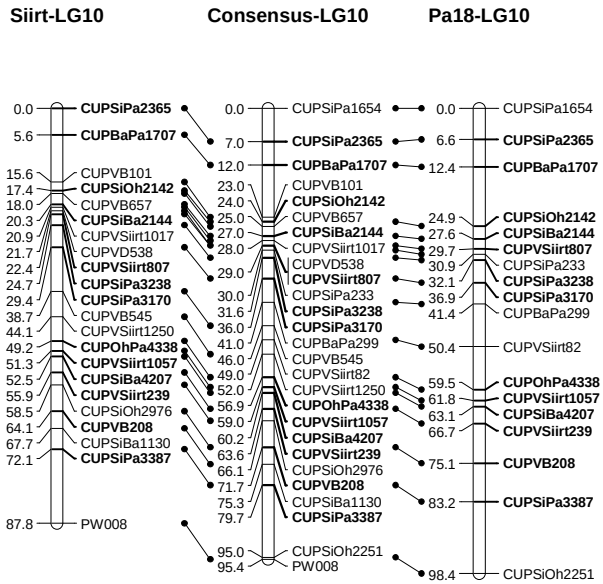


Figure 4.18. Parental and consensus maps of LG10 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

Table 4.20. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG10 map of 'Siirt x Pa-18' F1 population

No	Locus Name	Position	Segregation type	X2	Significance
1	CUPSiPa1654	0.0	nnxnp	0.79	-
2	CUPSiPa2365	7.0	abxcd	1.37	-
3	CUPBaPa1707	12.3	a0xcd	5.00	-
4	CUPVB101	22.6	lmx00	1.29	-
5	CUPSiOh2142	24.3	abxcd	4.20	-
6	CUPVB657	24.9	lmxll	1.40	-
7	CUPSiBa2144	27.1	abxcd	4.93	-
8	CUPVSiirt1017	27.7	lmx00	39.12	*******
9	CUPVD538	28.6	lmxll	3.17	*
10	CUPVSiirt807	29.3	efxeg	5.61	-
11	CUPSiPa233	30.2	nnxnp	0.13	-
12	CUPSiPa3238	31.6	efxeg	3.16	-
13	CUPSiPa3170	36.1	efxeg	6.76	*
14	CUPBaPa299	40.5	00xnp	0.03	-
15	CUPVB545	45.9	lmxll	3.23	*
16	CUPVSiirt82	48.6	nnxnp	0.54	-
17	CUPVSiirt1250	51.5	lmx00	0.40	-
18	CUPOhPa4338	56.9	abxcd	2.01	-
19	CUPVSiirt1057	59.0	abxcd	1.68	-
20	CUPSiBa4207	60.2	efxeg	2.71	-
21	CUPVSiirt239	63.6	abxcd	1.87	-
22	CUPSiOh2976	66.1	lmx00	0.66	-
23	CUPVB208	71.7	abxc0	2.52	-
24	CUPSiBa1130	75.3	lmxll	0.30	-
25	CUPSiPa3387	79.7	abxcd	1.34	-
26	CUPSiOh2251	95.0	nnxnp	1.18	-
27	PW008	95.4	lmxll	0.39	-

(*) $p<0.05$, (*******) $p<0.00005$.

The consensus map of LG10 had 27 SSR markers with a total length of 95.4 cM. There were 7 'abxcd', 1 'abxc0', 1 'a0xcd', 4 'efxeg', 5 'lmxll' 4 'lmx00', 4 'nnxnp' and 1 '00xnp' segregated markers in the consensus map. A total of 13 common markers were mapped in the LG10 consensus map of 'Siirt x Pa-18' F1 population. Four SSR markers had distorted segregation among 27 mapped SSR markers in the consensus map. The average distance between SSR markers was 3.5 cM and the average marker density was 0.28. The largest gap in LG10 consensus

map was between the CUPSiPa3387 and CUPSiOh2251 markers with 15.3 cM (Figure 4.18 and Table 4.20).

4.3.11. LG11 of 'Siirt x Pa-18' F1 Population

'Siirt' LG11 map was constructed by 14 markers with a total map length of 76.1 cM. The average distance between the markers was 5.4 cM and the average marker density was 0.18 markers per cM (supplementary file 1). There was a large gap near the centromere region 'Siirt' LG11 map with 21 cM length between the CUPSiBa1162 and CUPSiOh4084 markers (Figure 4.19). LG11 of 'Pa-18' genotype had 14 markers, and the total length was 72.4 cM. The average distance between SSR markers was 5.2 cM and the average marker density was 0.19 (supplementary file 2).

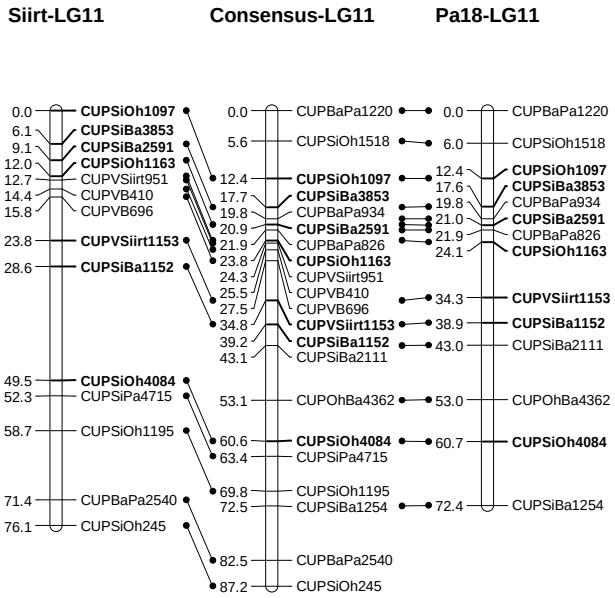


Figure 4.19. Parental and consensus maps of LG11 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

The consensus LG11 map had 21 SSR markers with a total length of 87.2 cM. The numbers of abxcd, lmxll, lmx00, nnxnp and 00xnp segregated markers were 7, 5, 2, 6, 1, respectively. So, a total of seven common markers were mapped in the LG11 consensus map. Two markers among 21 mapped markers were developed from this study and 17 mapped markers were from a study by Khodaeiaminjan et al (2017b). Of mapped 21 SSR markers, 7 markers showed segregation distortion. The average distance between SSR markers was 4.2 cM and the average marker density was 0.24 (Figure 4.19 and Table 4.21).

Table 4.21. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG11 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPBaPa1220	0.0	nnxnp	0.03	-
2	CUPSiOh1518	5.6	nnxnp	0.00	-
3	CUPSiOh1097	12.4	abxcd	5.37	-
4	CUPSiBa3853	17.7	abxcd	10.47	**
5	CUPBaPa934	19.8	nnxnp	2.35	-
6	CUPSiBa2591	20.9	abxcd	5.81	-
7	CUPBaPa826	21.9	nnxnp	2.57	-
8	CUPSiOh1163	23.8	abxcd	8.93	**
9	CUPVSiirt951	24.3	lmxll	3.45	*
10	CUPVB410	25.5	lmxll	0.03	-
11	CUPVB696	27.5	lmxll	3.59	*
12	CUPVSiirt1153	34.8	abxcd	4.89	-
13	CUPSiBa1152	39.2	abxcd	6.50	*
14	CUPSiBa2111	43.1	nnxnp	0.13	-
15	CUPOhBa4362	53.1	nnxnp	0.21	-
16	CUPSiOh4084	60.6	abxcd	7.49	*
17	CUPSiPa4715	63.4	lmxll	3.17	*
18	CUPSiOh1195	69.8	lmx00	1.03	-
19	CUPSiBa1254	72.5	00xnp	0.39	-
20	CUPBaPa2540	82.5	lmxll	0.07	-
21	CUPSiOh245	87.2	lmx00	0.67	-

(*) $p<0.05$, (**) $p<0.01$.

4.3.12. LG12 of 'Siirt x Pa-18' F1 Population

LG12 map of Siirt cultivar had 17 SSR markers with a total map length of 103.8 cM. The average distance between the markers was 6.1 cM and the average marker density was 0.16 markers per cM (supplementary file 1). There was a large gap between the CUPOhBa4344 and CUPSiOh1704 markers with 21 cM in 'Siirt' LG12 map (Figure 4.20). LG12 of 'Pa-18' genotype had 17 markers, and the total LG length was 73 cM. The average distance between the markers was 4.3 cM and the average marker density was 0.23 (supplementary file 2). The consensus LG12 map had 27 SSR markers with a total length of 105.7 cM. The numbers of abxcd, a0xcd, efxeg, lmxll, lmx00 and nnxnp segregations in the consensus map were 5, 1, 1, 8, 2 and 10 respectively. Of 27 mapped SSR markers in the consensus LG12, two SSR loci were developed in this study while 20 markers were from Khodaeiaminjan et al (2017b; Figure 4.20 and Table 4.22).

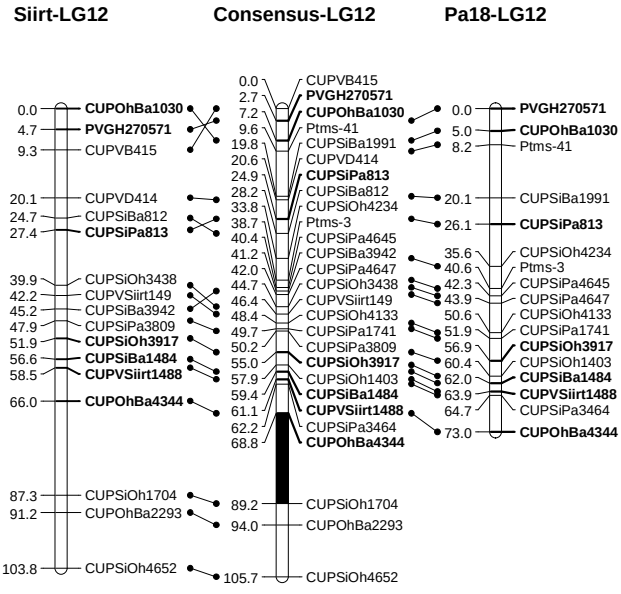


Figure 4.20. Parental and consensus maps of LG12 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

A total of 7 common markers were mapped in the consensus LG12 map of 'Siirt x Pa-18' F1 population. Among mapped 27 SSR markers in the consensus map, three markers had distorted segregation. The average distance between SSR markers was 3.9 cM and the average marker density was 0.26. There was a large gap between the CUPOhBa4344 and CUPSiOh1704 markers with 20.4 cM (Figure 4.20 and Table 4.22).

Table 4.22. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG12 map of 'Siirt x Pa-18' F1 population

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPVB415	0.0	lmx00	0.20	-
2	PVGH270571	2.7	efxeg	3.03	-
3	CUPOhBa1030	7.2	abxcd	1.67	-
4	Ptms-41	9.6	nnxnp	0.40	-
5	CUPSiBa1991	19.8	nnxnp	1,66	-
6	CUPVD414	20.6	lmxll	0.20	-
7	CUPSiPa813	24.9	abxcd	4.50	-
8	CUPSiBa812	28.2	lmxll	0.13	-
9	CUPSiOh4234	33.8	nnxnp	0.66	-
10	Ptms-3	38.7	nnxnp	0.00	-
11	CUPSiPa4645	40.4	nnxnp	0.52	-
12	CUPSiBa3942	41.2	lmxll	0.07	-
13	CUPSiPa4647	42.0	nnxnp	0.53	-
14	CUPSiOh3438	44.7	lmxll	0.41	-
15	CUPVSiirt149	46.4	lmx00	0.20	-
16	CUPSiOh4133	48.4	nnxnp	1.35	-
17	CUPSiPa1741	49.7	nnxnp	2.51	-
18	CUPSiPa3809	50.2	lmxll	0.07	-
19	CUPSiOh3917	55.0	abxcd	3.31	-
20	CUPSiOh1403	57.9	nnxnp	2.75	*
21	CUPSiBa1484	59.4	abxcd	3.48	-
22	CUPVSiirt1488	61.1	a0xcd	5.54	-
23	CUPSiPa3464	62.2	nnxnp	6.84	***
24	CUPOhBa4344	68.8	abxcd	3.33	-
25	CUPSiOh1704	89.2	lmxll	4.45	**
26	CUPOhBa2293	94.0	lmxll	0.13	-
27	CUPSiOh4652	105.7	lmxll	0.32	-

(*) $p<0.05$, (**) $p<0.01$, (***) $p<0.005$.

4.3.13. LG13 of 'Siirt x Pa-18' F1 Population

LG13 of 'Siirt' cultivar was constructed by 25 markers with a total map length of 96.8 cM. The average distance between the markers was 3.9 cM and the average marker density was 0.26 markers per cM (supplementary file 1). LG13 of 'Pa-18' genotype had 23 markers, and the total length was 95.4 cM. The average distance between SSR markers was 4.1 cM and the average marker density was 0.24 (supplementary file 2).

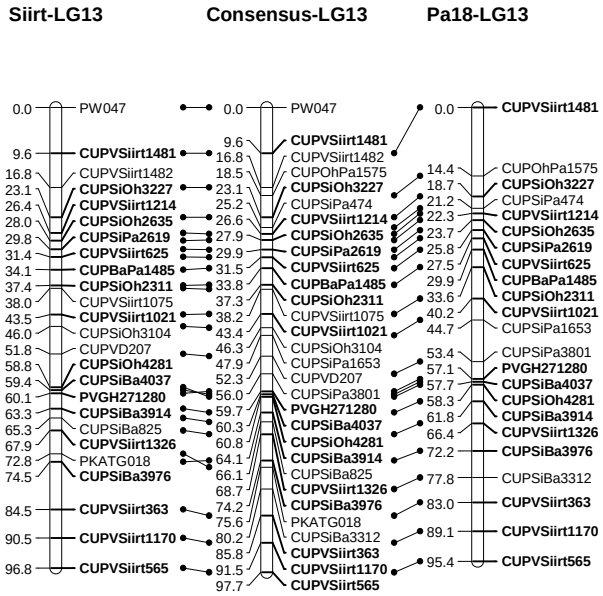


Figure 4.21. Parental and consensus maps of LG13 in 'Siirt x Pa-18' F1 population. The common markers are given in bold.

The consensus map of LG13 had 30 SSR markers with a total length of 97.7 cM. The numbers of abxcd, abxc0, efxeg, lmxll, lmx00 and nnxnp segregations in the consensus map were 12, 3, 3, 5, 2 and 5 respectively. So, a total of 30 common markers were mapped in the LG13 consensus map of 'Siirt x Pa-18' F1 population. Of mapped 30 SSR markers, only two SSRs were distorted markers. The average distance between SSR markers was 3.3 cM and the average marker

density was 0.31. There was no large gap in LG13 consensus map. The LG13 had the highest number (10 markers) of markers which developed in this study (Figure 4.21 and Table 4.23).

Table 4.23. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG13 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	PW047	0.0	lmxll	0,08	-
2	CUPVSiirt1481	9.6	abxc0	7.81	*
3	CUPVSiirt1482	16.8	lmxll	0.66	-
4	CUPOhPa1575	18.5	nnxnp	0.14	-
5	CUPSiOh3227	23.1	abxcd	0.89	-
6	CUPSiPa474	25.2	nnxnp	0.13	-
7	CUPVSiirt1214	26.6	abxc0	0.19	-
8	CUPSiOh2635	27.9	abxcd	0.16	-
9	CUPSiPa2619	29.9	abxcd	0.92	-
10	CUPVSiirt625	31.5	abxcd	1.30	-
11	CUPBaPa1485	33.8	abxcd	1.30	-
12	CUPSiOh2311	37.3	abxcd	0.56	-
13	CUPVSiirt1075	38.2	lmx00	0.04	-
14	CUPVSiirt1021	43.4	abxcd	1.10	-
15	CUPSiOh3104	46.3	lmxll	0.21	-
16	CUPSiPa1653	47.9	nnxnp	0.29	-
17	CUPVD207	52.3	lmxll	2.06	-
18	CUPSiPa3801	56.0	nnxnp	0.67	-
19	PVGH271280	59.7	efxeg	2.71	-
20	CUPSiBa4037	60.3	abxcd	2.14	-
21	CUPSiOh4281	60.8	abxc0	1.43	-
22	CUPSiBa3914	64.1	abxcd	3.16	-
23	CUPSiBa825	66.1	lmx00	1.83	-
24	CUPVSiirt1326	68.7	abxcd	3.10	-
25	CUPSiBa3976	74.2	efxeg	2.91	-
26	PKATG018	75.6	lmxll	3.33	*
27	CUPSiBa3312	80.2	nnxnp	0.13	-
28	CUPVSiirt363	85.8	efxeg	4.49	-
29	CUPVSiirt1170	91.5	abxcd	1.88	-
30	CUPVSiirt565	97.7	abxcd	3.21	-

(*) $p<0.05$.

4.3.14. LG14 of 'Siirt x Pa-18' F1 Population

'Siirt' LG14 map was constructed by 16 SSR markers. A total map length was 117.1 cM with an average of 7.3 cM distance between the markers, and the average marker density was 0.14 markers per cM (supplementary file 1). 'Pa-18' LG14 map had 18 markers, and the total LG length was 115.5 cM. The average distance between the markers was 6.4 cM and the average marker density was 0.16 (supplementary file 2).

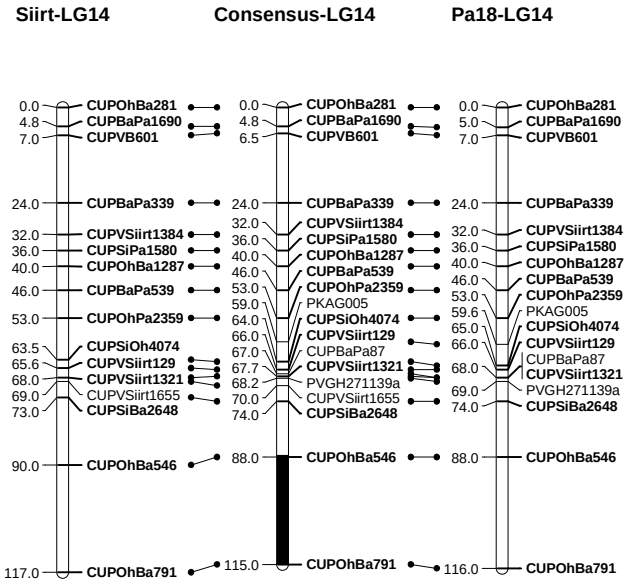


Figure 4.22. Parental and consensus maps of LG14 in 'Siirt x Pa-18' F1 population. The common markers are given in bold. The black segments represent the gaps ≥20 cM.

Nineteen markers were mapped in the consensus LG14 with a total length of 114.9 cM. The numbers of abxcd, abxc0, a0xcd, efxeg, lmxll and nnxnp segregations in the consensus map were 9, 1, 1, 4, 1 and 3 respectively. A total of 15 common markers were mapped in the LG14 consensus map. Among mapped 19 SSR markers in the consensus map, only the CUPSiPa1580 marker had distorted segregation. The average distance between SSR markers was 6.0 cM and the

average marker density was 0.17. The largest gap in LG14 consensus map was between CUPOhBa546 and CUPOhBa791 with 27.0 cM. In the consensus LG14 map, four markers were developed in this study and 12 markers were developed by Khodaeiaminjan et al (2017b; Figure 4.22 and Table 4.24).

Table 4.24. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG14 map of 'Siirt x Pa-18' F1 population

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPOhBa281	0.0	a0xcd	2.39	-
2	CUPBaPa1690	4.8	efxeg	1.94	-
3	CUPVB601	6.5	abxcd	2.26	-
4	CUPBaPa339	24.0	abxcd	0.97	-
5	CUPVSiirt1384	31.9	abxcd	4.71	-
6	CUPSiPa1580	35.8	efxeg	6.90	*
7	CUPOhBa1287	39.5	efxeg	4.13	-
8	CUPBaPa539	45.9	abxcd	1.50	-
9	CUPOhPa2359	52.7	abxcd	2.67	-
10	PKAG005	59.1	nnxnp	0.65	-
11	CUPSiOh4074	64.0	abxcd	2.58	-
12	CUPVSiirt129	65.7	abxcd	1.34	-
13	CUPBaPa87	67.0	nnxnp	1.56	-
14	CUPVSiirt1321	67.7	abxcd	1.18	-
15	PVGH271139a	68.2	nnxnp	0.98	-
16	CUPVSiirt1655	69.6	lmxll	0.13	-
17	CUPSiBa2648	73.8	efxeg	3.20	-
18	CUPOhBa546	87.6	abxcd	0.60	-
19	CUPOhBa791	114.9	abxc0	1.17	-

(*) $p<0.05$.

4.3.15. LG15 of 'Siirt x Pa-18' F1 Population

Twenty-three markers were mapped in 'Siirt' LG15. The total map length was 80.6 cM and the average marker density was 0.29 markers per cM. The average distance between the markers was 3.5 cM (supplementary file 1). LG15 of 'Pa-18' genotype had 17 markers, and the total LG length was 49.6 cM. The average distance between the markers was 2.9 cM and the average marker density was 0.34 (supplementary file 2).

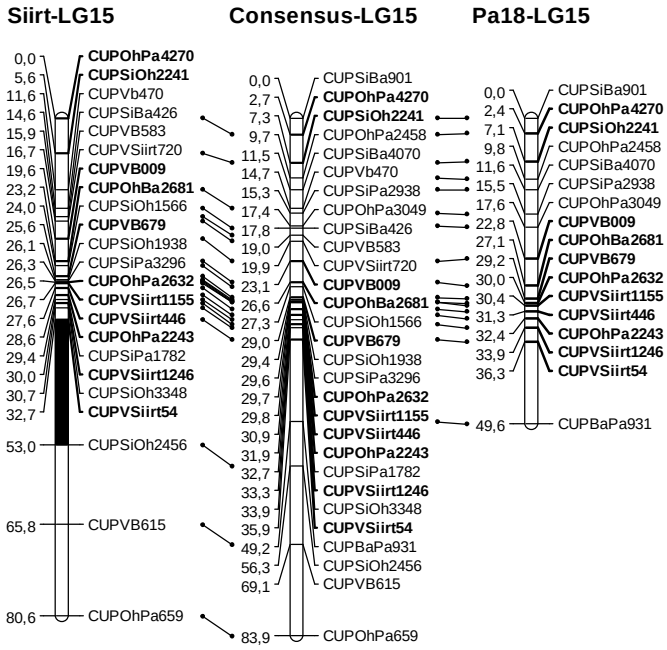


Figure 4.23. Parental and consensus maps of LG15 in 'Siirt x Pa-18' F1 population. The common markers are given in bold. The black segment represents the gap ≥20 cM.

The consensus map of LG15 had 29 SSR markers with a total length of 83.9 cM. The numbers of abxcd, efxeg, lmxll, lmx00 and nnxnp segregations in the consensus map were 9, 2, 10, 2 and 6, respectively. A total of 11 common markers were mapped in the LG15. Twenty markers had distorted segregation among mapped 29 SSR markers in the consensus map. The average distance between SSR

markers was 2.9 cM and the average marker density was 0.35. The largest gap in LG15 consensus map was between CUPVB615 and CUPOhPa659 markers with 14.8 cM (Figure 4.23 and Table 4.25). From 29 mapped markers in the consensus of LG15, five markers were developed in this study and 19 markers from Khodaeiaminjan et al (2017a; Figure 4.23 and Table 4.25).

Table 4.25. Locus names, positions (cM), segregation types, chi-square values (X^2) and significant levels of distorted SSR loci in the consensus LG15 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Segregation type	X^2	Significance
1	CUPSiBa901	0.0	nnxnp	0	-
2	CUPOhPa4270	2.7	abxcd	6.14	-
3	CUPSiOh2241	7.3	abxcd	8.50	**
4	CUPOhPa2458	9.7	nnxnp	0,08	-
5	CUPSiBa4070	11.5	nnxnp	0,03	-
6	CUPVb470	14.7	lmx00	7.14	***
7	CUPSiPa2938	15.3	nnxnp	0.52	-
8	CUPOhPa3049	17.4	nnxnp	0.83	-
9	CUPSiBa426	17.8	lmxll	8.71	****
10	CUPVB583	19.0	lmxll	10.95	*****
11	CUPVSiirt720	19.9	lmxll	7.19	***
12	CUPVB009	23.1	abxcd	8.55	**
13	CUPOhBa2681	26.6	abxcd	9.88	**
14	CUPSiOh1566	27.3	lmxll	4.65	**
15	CUPVB679	29.0	abxcd	12.98	****
16	CUPSiOh1938	29.4	lmxll	8.39	****
17	CUPSiPa3296	29.6	lmxll	6.95	***
18	CUPOhPa2632	29.7	abxcd	9.56	**
19	CUPVSiirt1155	29.8	abxcd	8.89	**
20	CUPVSiirt446	30.9	efxeg	10.39	**
21	CUPOhPa2243	31.9	abxcd	12.06	***
22	CUPSiPa1782	32.7	lmxll	9.80	****
23	CUPVSiirt1246	33.3	abxcd	16.82	*****
24	CUPSiOh3348	33.9	lmx00	8.13	****
25	CUPVSiirt54	35.9	efxeg	21.4	*******
26	CUPBaPa931	49.2	nnxnp	0.82	-
27	CUPSiOh2456	56.3	lmxll	7.14	***
28	CUPVB615	69.1	lmxll	2.70	-
29	CUPOhPa659	83.9	lmxll	0.53	-

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

4.4. Linkage Map Construction With Common Markers in 'Siirt X Pa-18' F1 Population

After constructing consensus and parental maps, a reference map with common markers was generated (Figure 4.24). A total of 203 SSR markers were mapped: 134 of them had ab×cd, 26 were abxc0, 38 were ef×eg and 5 were hk×hk segregating markers along with 15 linkage groups (Table 4.26). The total map length was 1,223 cM, with a 6.0 cM average distance between markers. Lengths of linkage groups ranged from 18.4 cM in LG1 to 131.9 cM in LG8. The average linkage-group length was 81.5 cM, and the average number of markers per linkage group was 13.5. The highest average distance between SSR markers were observed in LG6 with 11.3 cM and the lowest one was in LG1 with 1.8 cM. LG9 had the highest number of common markers (28), while LG3, LG11, LG12 had the lowest numbers (7).

Table 4.26. Segregation types, length, number of loci, average distance between SSR markers and marker density of each LG in map with common markers in 'Siirt x Pa-18' F1 population.

LG	abxcd	abxc0	a0xcd	efxeg	hkxhk	Number of loci	Length	Average marker distance	Marker density
1	6	2	0	2	0	10	**18.4**	**1.8**	0.54
2	8	2	0	2	2	14	100.8	7.2	0.14
3	6	0	0	1	0	**7**	23.4	3.3	0.30
4	8	2	1	5	0	16	117.9	7.4	0.14
5	15	1	1	2	1	20	100.5	5.0	0.20
6	6	2	1	2	0	11	124.2	**11.3**	0.09
7	7	3	0	1	2	13	84.2	6.5	0.15
8	7	2	0	4	0	13	80.6	6.2	0.16
9	22	0	1	5	0	**28**	**131.9**	4.7	0.21
10	7	1	1	4	0	13	76.4	5.9	0.17
11	7	0	0	0	0	**7**	50.0	7.1	0.14
12	5	0	1	1	0	**7**	69.3	9.9	0.10
13	12	3	0	3	0	18	94.2	5.2	0.19
14	9	1	1	4	0	15	118	7.9	0.13
15	9	0	0	2	0	11	33.2	3.0	0.33
Total	**134**	**19**	**7**	**38**	**5**	**203**	**1,223**		
Average	**10.66**	**1.8**	**0.5**	**2.53**	**0.33**	**13.5**	**81.5**	**6**	**0.17**

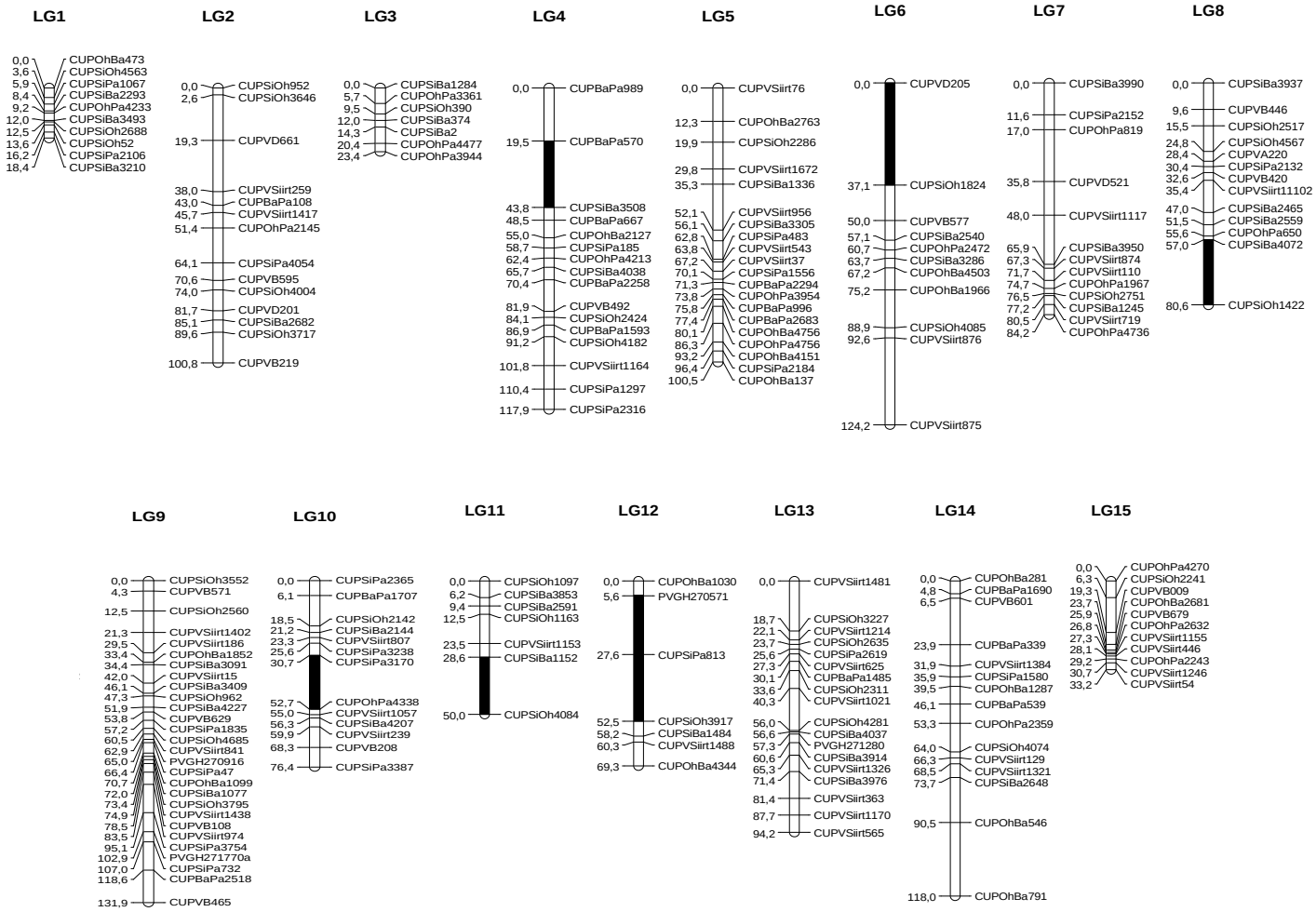


Figure 4.24. Reference map in pistachio using ‘Siirt x Pa-18’ F1 population. The black segments represent the gaps ≥20 cM.

4.5. Evaluation of SSR-Based Genetic Maps in Siirt × Pa-18 F1 Population

In this study, a total of 388 SSR loci were mapped in Siirt x Pa-18 F1 population. A total of 203 SSRs (52%) were heterozygous in both parents with a segregation ratio of either 1:1:1:1 or 1:2:1. A total of 103 (27%) SSR markers were heterozygous in female parent (Siirt) and homozygous in male parent (Pa-18) having lmxll segregation type. In addition, 82 markers (21%) had nnxnp segregation type which was heterozygous in male parent and homozygote in female parent with a Mendelian ratio of 1:1 (Table 4.27).

Table 4.27. Numbers of mapped markers and null alleles

Total	Mapped markers	Mapped markers with null allele
Total number of mapped markers	388	62 (16%)
Number of markers (1:1:1:1,1:2:1) segregating in both parents and consensus	203 (52%)	27 (43%)
Number of markers (1:1) segregating in 'Siirt'	103 (27%)	31 (50%)
Number of markers (1:1) segregating in 'Pa-18'	82 (21%)	4 (7%)

The segregation types of the 203 mapped common markers were as follows: 160 'abxcd', 38 'efxeg' and 5 'hkxhk'. 'Siirt' map included 306 (78%) markers, and 'Pa-18' map contained 285 (73%) markers, while 386 SSR markers were mapped in the consensus map. The information of segregation types of SSR markers in all four maps are presented in Table 4.28. According to the consensus map, CUPSiBa3210 and CUPSiOh952 markers were assigned in LG1 and LG2, respectively, but they were eliminated due to their high mean chisquare distribution values during the reference map construction.

In this study the frequency of markers with null allele were calculated (Table 4.27). Null alleles are alleles that for various reasons fail to amplify in a

PCR assay. The presence of null alleles in microsatellite data is known to bias the genetic parameter estimates (Dabrowski et al, 2015). When an allele fails to amplify in a PCR assay there are two possible outcomes; 1) in the case of a heterozygous locus, it appears to be homozygous, and 2) in the case of a homozygous locus, it remains undetected (Oddou-Muratorio et al. 2009). From a total of 203 common markers, 27 (43%) markers showed null allele in their segregations. Among markers which heterozygous in female parent (Siirt) and homozygous in male parent (Pa-18) and vice versa, just 35 (54%) markers showed null alleles in their segregations. Of 103 markers with heterozygosity patern in female parent and 82 markers with hetrozygosity patern in male parent, 31 (50%) and 4 (7%) markers were showed null alleles in their segregations, respectively (Table 4. 27). The high differences rations of null alleles in Siirt and Pa-18 map were related to the distances between *P.vera* and monoecious *P. atlantica*. In this study the null alleles were determined in various region of different linkage groups. The highest number of null allele was detected in LG1, while the LG3, LG14 and LG15 were the lowest. The association between the presence of null alleles and highly variable flanking regions has been demonstrated repeatedly in molecular studies, and several studies have suggested that the sequences flanking microsatellites may be less stable than those in other genomic regions (Angers and Bernatchez 1997; Grimaldi and Crouau-Roy 1997; Meglecz et al. 2004).

Table 4.28. The segregation types of SSR markers in the constructed four maps.

Genetic maps	Loci	abxcd	abxc0	a0xcd	efxeg	hkxhk	lmxll	lmx00	nnxnp	00xnp
All maps	388	134	19	7	38	5	72	31	78	4
Consensus map	386	133	18	7	38	5	72	31	78	4
Female map	306	134	19	7	38	5	72	31	-	-
Male map	285	134	19	7	38	5	-	-	78	4
Reference map	203	134	19	7	38	5	-	-	-	-

In this study, SSR primer pairs from different studies in the literature were used. Ahmad et al. (2003) developed 14 SSRs and two were mapped. One SSR markers were mapped from Albaladejo et al (2008), who developed 8 SSR primer pairs. A total of 27 SSRs were generated by Arabnezhad et al (2011), and four SSRs were mapped in this study. Chen et al (2012) developed 14 SSR primer pairs and 2 markers were mapped. A total of 14 SSR markers were mapped from Zaloglu et al (2015), who published 59 genomic SSRs. Thirty-five SSRs were mapped from Topçu et al (2016), who developed 84 SSRs in *P. vera*. A total of 19 EST-SSR markers were mapped from Khodaeiaminjan et al (2017a), who generated 170 SSRs from NCBI EST database in *Pistacia* A total of 249 SSRs were mapped from Khodaeiaminjan et al (2017b), who did the last SSR development study in pistachio and in this study 62 SSRs were mapped (Table 4.29).

Table 4. 29. The numbers of published and mapped SSR markers from different studies in Siirt x Pa-18 F1 population.

Reference	**Origin**	**Acronyms**	**Number of SSR primers**	**Mapped Markers**
Ahmad et al (2003)	*P. vera* cv	Pmts	14	2
Albaladejo et al (2008)	*P. lentiscus*	Pislen	8	1
Arabnezhad et al (2011)	*P. khinjuk*	PKAG	27	4
Chen et al (2012)	*P. weinmannifolia*	PW	14	2
Zaloglu et al (2015)	*P. vera* cv 'Siirt'	CUPV	59	14
Topçu et al (2016)	*P. vera* cv 'Siirt'	CUPV	84	35
Khodaeiamınjan et al (2017a)	NCBI database	PVGH, PAEF, PVJQ, PVJN, PVDQ, PVFJ	170	19
Khodaeiamınjan et al (2017b)	*P. vera* .cvs Siirt, Ohadi and Bağyolu, and *P. atlantica*	CUP	618	249
In this study	*P. vera* cv 'Siirt'	CUPVSiirt	950	62
Total			**1200**	**388**

This study describes construction of the first SSR-based genetic linkage map using an inter-specific cross between *P. vera* 'Siirt' x *P. atlantica* Desf. (monoecious Pa-18 genotype). There are two genetic mapping studies in the literature for *Pistacia*. The first genetic map in *Pistacia* was constructed by Turkeli and Kafkas (2013) with 321 ISSR, SRAP, AFLP markers,. This study used the same inter-specific cross, however the progenies were different due to death of several tens of F1 progenies. The second linkage map was constructed by Khodaeiaminjan et al (2017b) who mapped 385 SSRs using 122 progenies from 'Siirt x Bağyolu' intra-specific cross.

In this study, a total of 386 markers were successfully mapped in the consensus map of 'Siirt x Pa-18' F1 population along with 15 linkage groups. However, Khodaeiaminjan et al (2017b) was reported 385 mapped markers in

consensus map of ‘Siirt x Bağyolu’ population. The number of mapped common markers in this study was 201 (52.1%), whereas Khodaeiaminjan et al (2017b) mapped 273 (70.9%) common markers. Intra-specific population showed higher number of common markers than inter-specific population. Consequently, allelic differences in inter-specific population was higher than inter-specific population. During meiosis, homologous chromosomes pairs in inter-specific population exchange some of their genetic information which arising the genetic recombination and allele differences frequency. In inter-specific population, higher number of differences in SSR primer flanking regions can be reason of less number of common markers.

In the consensus map, the total map length was 1,486.1 cM with an average marker distance of 3.9 cM in this study. The result were similar with Khodaeiaminjan et al (2017b) differences were mainly in LG9. The differences in length and number of markers in linkage groups could be related to differences of male parents. The LG length varied from 41.6 cM (LG1) to 137.1 cM (LG2) in current study, while Khodaeiaminjan et al (2017b) reported LG length between 61.3 cM (LG8) and 131 cM (LG12). The average LG length was 99.1 cM, while it was 100.7 cM in Khodaeiaminjan et al (2017b). The average distance between the markers varied from 1.5 cM (LG1) to 6.0 cM (LG14), whereas Khodaeiaminjan et al (2017b) reported them from 2.8 (LG3) to 5.1 (LG15). In this study, the highest number of markers was mapped in LG9 (32 markers) which is similar to Khodaeiaminjan et al (2017b) results with 40 markers (LG9). Less number of markers were determined in LG8 and LG14 with 19 markers, while Khodaeiaminjan reported in LG11 (16 markers) that revealed in different linkage group in this study. Average marker density changed between 0.17 (LG14) and 0.67, whereas it changed between 2.8 (LG3) to 5.1 (LG15) in Khodaeiaminjan et al (2017b).

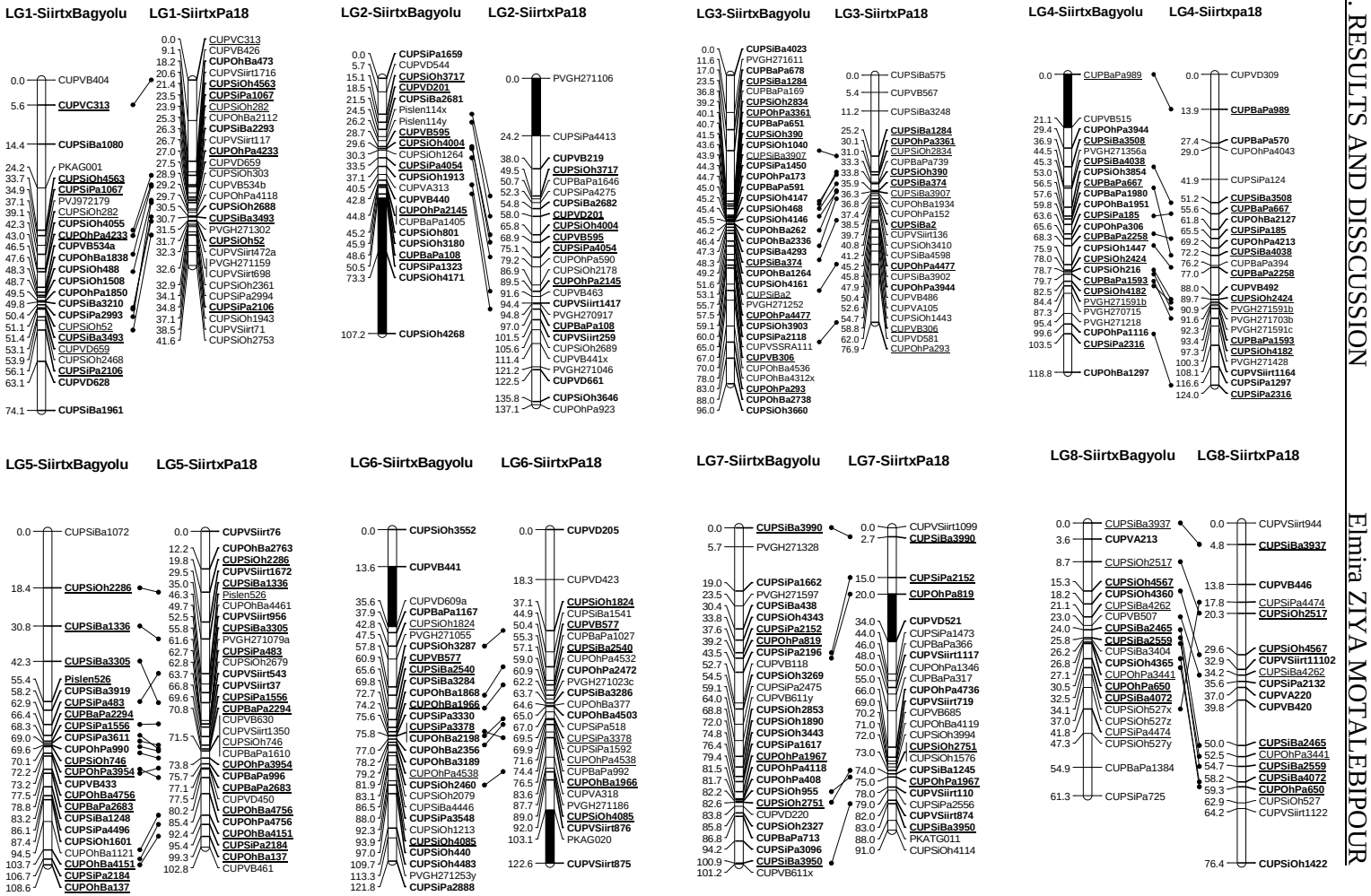
Table 4.30. Summary of the mapped SSR markers in consensus map of 'Siirt x Pa-18' F1 population

LG	Segregation								Number of loci	Length	Average marker distance	Marker density
	abxcd	abxc0	a0xcd	efxeg	hkxhk	lmxll	lmx00	nnxnp	00xnp			
1	5	2	0	2	0	7	5	6	1	28	41.6	1.5	0.67
2	8	1	0	2	2	3	2	7	0	25	137.1	5.5	0.18
3	6	0	0	1	0	9	2	7	0	25	76.9	3.1	0.33
4	8	2	1	5	0	5	0	2	1	24	124	5.2	0.19
5	15	1	1	2	1	6	2	2	0	30	102.8	3.4	0.29
6	6	2	1	2	0	4	1	9	0	25	122.6	4.9	0.2
7	7	3	0	1	2	1	3	8	0	25	90.5	3.6	0.28
8	7	2	0	4	0	0	3	3	0	19	76.4	4.0	0.25
9	22	0	1	5	0	3	1	0	0	32	129.4	4.0	0.25
10	7	1	1	4	0	5	4	4	1	27	95.4	3.5	0.28
11	7	0	0	0	0	5	2	6	1	21	87.2	4.2	0.24
12	5	0	1	1	0	8	2	10	0	27	105.7	3.9	0.26
13	12	3	0	3	0	5	2	5	0	30	97.7	3.3	0.31
14	9	1	1	4	0	1	0	3	0	19	114.9	6.0	0.17
15	9	0	0	2	0	10	2	6	0	29	83.9	2.9	0.35
Total	133	18	7	38	5	72	31	78	4	386	1,486.10		
Average	8.9	1.2	0.5	2.5	0.3	4.8	2.1	5.2	0.3	25.7	99.1	3.9	0.26

The comparison of order of the markers in the linkage groups of both maps (in this study and Khodaeiaminjan et al, 2017b) was made and they were highly similar which shows reliability of the maps. In both populations, linkage maps showed high degree of collinearity with each other. However, several minor differences in the marker order were also detected. For example, CUPOhPa3944 marker was mapped in the top of LG4 in the maps constructed by Khodaeiaminjan et al. (2017b), while the marker was mapped in the bottom of LG3 in this study. In this study, CUPOhPa4118 marker was mapped in LG1, whereas it was mapped in LG7 in study by Khodaeiaminjan et al. (2017b). For another example, CUPSiOh3552 marker was mapped in LG9 in this study, while it was mapped in LG6 constructed by Khodaeiaminjan et al. (2017b). Totally, there were 148 shared SSRs between two different populations. The shared markers from each linkage group are presented in Table 4. 31 and Figure 4.25. These markers can be informative for the further studies in the genus *Pistacia*.

Table 4.31. The number of shared SSRs between current study and the study performed by Khodaeiaminjan et al, (2017b)

LG	Current study	Khodaeiaminjan et al, (2017b)	Shared markers
1	28	24	9
2	25	22	7
3	25	35	10
4	24	24	12
5	30	23	14
6	25	28	8
7	25	28	7
8	19	20	11
9	32	40	14
10	27	27	9
11	21	16	8
12	27	33	7
13	30	22	8
14	19	21	11
15	29	22	13
Total	386	385	148


LG1-SiirtxBagyolu
LG1-SiirtxPa18
LG2-SiirtxBagyolu
LG2-SiirtxPa18
LG3-SiirtxBagyolu
LG3-SiirtxPa18
LG4-SiirtxBagyolu
LG4-Siirtxpa18
LG5-SiirtxBagyolu
LG5-SiirtxPa18
LG6-SiirtxBagyolu
LG6-SiirtxPa18
LG7-SiirtxBagyolu
LG7-SiirtxPa18
LG8-SiirtxBagyolu
LG8-SiirtxPa18

Countined

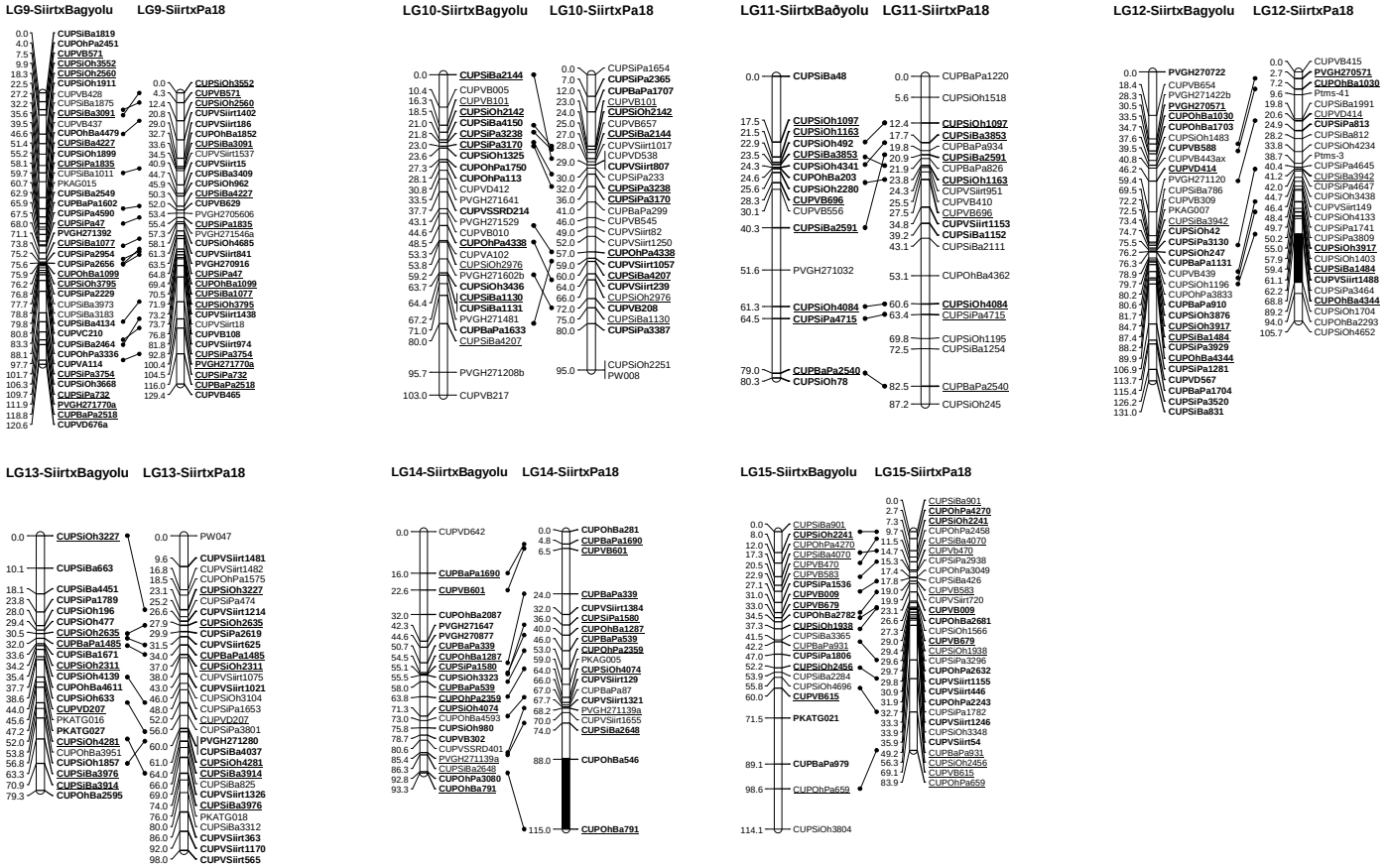


Figure 4.25. Consensus map in pistachio using 'Siirtx Bagyolu' (Left side) and 'Siirt x Pa-18' (Right site) F1 population. Bold letters indicate the common-segregation microsatellite loci in each study and the underline letters indicate the shared markers between two study. Map distances calculated between markers are expressed in Kosambi function (cM) on the left side of each bar and the marker names are given on the right side of each bar. The black segments represent the gaps >20 cM

A total of 306 markers were successfully mapped in Siirt female parent along with 15 linkage groups. The number of mapped common markers was 203 (66.3%) in female map, and the total map length was 1,410.4 cM, with an average marker distance of 4.6 cM. The LG length varied from 38.7 cM (LG1) to 124.8 cM (LG2). The average length of the linkage groups was 94.0 cM. The average marker distance varied from 1.8 cM (LG1) to 7.7 cM (LG6). The number of markers in 15 LGs were changed between 14 (LG11) and 32 (LG9). The average marker density was between 0.14 (LG14) and 0.57 (LG1; Table 4.32). Khoadeiaminjan et al (2017b) reported a total of 334 SSR markers in 'Siirt' genetic map spanning 1,423.3 cM length with an average marker distance of 4.3. The length of 'Siirt' genetic linkage map was similar to this study, but average marker density in Khodaeiaminjan et al (2017b) was better than this study. Furthermore, the constructed genetic linkage 'Siirt' map by Turkeli and Kafkas (2013) with ISSR, AFLP, SRAP markers in 'Siirt x Pa-18' F1 population had 17 LGs with 1237.3 cM and the average markers distance was 7.50 cM. The length of 'Siirt' map of Turkeli and Kafkas (2013) study was shorter and the average marker distance was higher than current study.

Table 4 32. Summary of the mapped SSR markers in 'Siirt' female map of 'Siirt x Pa-18' F1 population

LG	Segregation							Number of loci	Length	Average marker distance	Marker density
	abxcd	abxc0	a0xcd	efxeg	hkxhk	lmxll	lmx00				
1	6	2	0	2	0	7	5	22	38.7	1.8	0.57
2	8	2	0	2	2	3	2	19	98.6	5.2	0.19
3	6	0	0	1	0	9	2	18	71.5	4.0	0.25
4	8	2	1	5	0	5	0	21	124.8	5.9	0.17
5	15	1	1	2	1	6	2	28	103.2	3.7	0.27
6	6	2	1	2	0	4	1	16	123.2	7.7	0.13
7	7	3	0	1	2	1	3	17	80.9	4.8	0.21
8	7	2	0	4	0	0	3	16	77.9	4.9	0.21
9	22	0	1	5	0	3	1	32	129.4	4.0	0.25
10	7	1	1	4	0	5	4	22	87.8	4.0	0.25
11	7	0	0	0	0	5	2	14	76.1	5.4	0.18
12	5	0	1	1	0	8	2	17	103.8	6.1	0.16
13	12	3	0	3	0	5	2	25	96.8	3.9	0.26
14	9	1	1	4	0	1	0	16	117.1	7.3	0.14
15	9	0	0	2	0	10	2	23	80.6	3.5	0.29
Total	134	19	7	38	5	72	31	306	1,410.40		
Average	8.9	1.3	0.5	2.5	0.3	4.8	2.1	20.4	94	4.6	0.22

In Pa-18 male parent, 285 markers were successfully mapped along with 15 linkage groups. The number of markers in 15 LGs were changed between 14 (LG11) and 28 (LG9). The number of mapped common markers was 203 (66.3%), and the total map length was 1,362.5 cM with an average marker distance of 4.8 cM. The LG length varied from 23.4 cM (LG1) to 140.7 cM (LG2). The average length of the linkage groups was 90.8 cM. The average marker distance varied from 1.4 cM (LG1) to 6.7 cM (LG2). The average marker density was between 0.16 (LG4, 6, 14) and 0.73 (LG1; Table 4.33). The constructed genetic linkage male maps by Turkeli and Kafkas (2013) with ISSR, AFLP, SRAP markers in 'Siirt x Pa-18' F1 population had 19 LGs in Pa-18 map. The Pa-18 map had 1337.5 cM with 8.57 cM distance between markers. Current study constructed with similar length of Pa-18 map. Khodaeiaminjan et al (2017b) mapped a total of 343 markers in male (Bağyolu) genotype. 'Bağyolu' genetic map had 1,453.4 cM length with a mean marker density of 4.2 cM.

Table 4.33. Summary of the mapped SSR markers in 'Pa-18' male map of 'Siirt x Pa-18' F1 population

LG	Segregation							Number of loci	Length	Average marker distance	Marker density
	abxcd	abxc0	a0xcd	efxeg	hkxhk	nnxnp	00xnp				
1	6	2	0	2	0	6	1	17	23.4	1.4	0.73
2	8	2	0	2	2	7	0	21	140.7	6.7	0.15
3	6	0	0	1	0	7	0	14	54.7	3.9	0.26
4	8	2	1	5	0	2	1	19	116.7	6.1	0.16
5	15	1	1	2	1	2	0	22	99.7	4.5	0.22
6	6	2	1	2	0	9	0	20	123.2	6.2	0.16
7	7	3	0	1	2	8	0	21	88.9	4.2	0.24
8	7	2	0	4	0	3	0	16	79	4.9	0.2
9	22	0	1	5	0	0	0	28	131.9	4.7	0.21
10	7	1	1	4	0	4	1	18	98.4	5.5	0.18
11	7	0	0	0	0	6	1	14	72.4	5.2	0.19
12	5	0	1	1	0	10	0	17	73	4.3	0.23
13	12	3	0	3	0	5	0	23	95.4	4.1	0.24
14	9	1	1	4	0	3	0	18	115.5	6.4	0.16
15	9	0	0	2	0	6	0	17	49.6	2.9	0.34
Total	134	19	7	38	5	78	4	285	1,362.50		
Average	8.9	1.3	0.5	2.5	0.3	5.2	0.3	19	90.8	4.8	0.21

Summary of the mapped SSR markers in consensus, female, male and reference maps of ‘Siirt x Pa-18’ F1 population are presented in Table 4.34. A total of 203 SSRs were successfully mapped in reference map of ‘Siirt x Pa-18’ F1 population using only coomon SSR markers in 15 linkage groups. The final reference genetic linkage map was 1,223 cM, consisting of 15 linkage groups with an average of 6.0 cM. The length of LGs ranged from 18.4 cM (LG1) to 131.9 cM (LG9). The highest SSR markers were mapped in LG9 with 28 markers while the lowest number of markers was linked in LG11 with seven markers. In current and Khodaeiaminjan et al (2017b) study the highest number of common markers was identified in LG9.

Table 4. 34. Summary of the mapped SSR markers in Consensus, female, male and reference map of 'Siirt x Pa-18' F1 population

	Consesus				Siirt				Pa-18				Reference			
LG	Length	Number of loci	Average marker distance	Marker density	Length	Number of loci	Average marker distance	Marker density	Length	Number of loci	Average marker distance	Marker density	Length	Number of loci	Average marker distance	Marker density
1	41.6	28	1.5	0.67	38.7	22	1.8	0.57	23.4	17	1.4	0.73	18.4	10	1.8	0.54
2	137.1	25	5.5	0.18	98.6	19	5.2	0.19	140.7	21	6.7	0.15	100.8	14	7.2	0.14
3	76.9	25	3.1	0.33	71.5	18	4.0	0.25	54.7	14	3.9	0.26	23.43	7	3.3	0.30
4	124	24	5.2	0.19	124.8	21	5.9	0.17	116.7	19	6.1	0.16	117.9	16	7.4	0.14
5	102.8	30	3.4	0.29	103.2	28	3.7	0.27	99.7	22	4.5	0.22	100.5	20	5.0	0.20
6	122.6	25	4.9	0.20	123.2	16	7.7	0.13	123.2	20	6.2	0.16	124.2	11	11.3	0.09
7	90.5	25	3.6	0.28	80.9	17	4.8	0.21	88.9	21	4.2	0.24	84.2	13	6.5	0.15
8	76.4	19	4.0	0.25	77.9	16	4.9	0.21	79.0	16	4.9	0.20	80.6	13	6.2	0.16
9	129.4	32	4.0	0.25	129.4	32	4.0	0.25	131.9	28	4.7	0.21	131.9	28	4.7	0.21
10	95.4	27	3.5	0.28	87.8	22	4.0	0.25	98.4	18	5.5	0.18	76.4	13	5.9	0.17
11	87.2	21	4.2	0.24	76.1	14	5.4	0.18	72.4	14	5.2	0.19	50.0	7	7.1	0.14
12	105.7	27	3.9	0.26	103.8	17	6.1	0.16	73.0	17	4.3	0.23	69.3	7	9.9	0.10
13	97.7	30	3.3	0.31	96.8	25	3.9	0.26	95.4	23	4.1	0.24	94.2	18	5.2	0.19
14	114.9	19	6.0	0.17	117.1	16	7.3	0.14	115.5	18	6.4	0.16	118.0	15	7.9	0.13
15	83.9	29	2.9	0.35	80.6	23	3.5	0.29	49.6	17	2.9	0.34	33.2	11	3.0	0.33
Total	1486.1	386			1410.4	306			1362.5	285			1223.0	203		
Avrage	99.1	25.7	3.9	0.26	94.0	20.4	4.6	0.22	90.8	19	4.8	0.21	81.5	13.5	6.0	0.17

Comparison of the female, consensus and male maps in this study showed that markers relative position confirms the robustness of the linear order of the markers and the perfect co-linearity of the genome. Only one large inversion was observed in LG7. Chromosomal inversions which couse recombination in the inverted region are widely recognized in insects, plants, bacteria, and humans (Hoffmann et al, 2004). In this study, during the evolution and divergence of *Pistacia* genus, a large region of inversions may have occurred in LG7 which can be explained by using an inter-specific cross *P. vera* cv. Siirt and monoecious *P. atlantica* in this study. Paracentric inversion on the long arm of linkage group 7 has not been observed in the study performed by Khodaeiaminjan et al (2017b) who used intra-specific F1 population.

4.6. Distribution Of Distorted Markers

Distribution of segregation distortion are also seen in plants genome like as animals. Their function is guided by competition among gametes for partial fertilization or abortion of female or male gametes or zygotes. Competition may occur due to gametophyte genes expressed in the haploid gamete resulting in distorted segregation ratios (Kumar and Kumar 2015). The order of local marker and the distance of linkage can be estimated in the influence of distorted markers. (Zhu et al, 2007) These markers may slightly have effects on QTL mapping (Xu 2008; Zhang et al, 2010).

In the current study, 100 markers (25.9 %) on the consensus map showed segregation distortion (SD). Khodaeiaminjan et al (2017b) reported 90 skewed markers (23.3%) on the consensus map of *Pistacia* genus. The percentage of segregation distortion in the ‘Siirt’ map was 30.4% (93 markers) in this study. The results were higher than Khodaeiaminjan et al (2017b) study and similar with the results reported by Turkeli and Kafkas (2013). The rate of segregation distortion in the ‘Pa-18’ map was 23.9 (68 markers, Table 4.35) and it was lower than previous study by Turkeli and Kafkas (2013). The skewed markers in this study were

unequally distributed on the 15 LGs. The highest percentage of SD in this study was determined in LG15 (%69.0) and followed by LG2 (%44.0), LG5 (%43.3), LG8 (%42.1) and LG6 (%40.0). In the study of Khodaeaminjan et al,. (2017b) the highest percentage of SD in their map was determined in LG8 (%55.0) and in LG2 (%50.0). From a total of 148 shared markers, 33 markers showed SD in Khodaeiaminjan et al (2017b), while 39 markers showed SD in this study, and only 5 markers were common between two populations. To understand possible causes of SD, we analyzed the distorted markers using the allelic and zygotic SD tests as described by Li et al (2011). As shown in supplementary file 3, among the 100 distorted markers, only three (CUPBaPa989, CUPVD205 and CUPVSiirt875) showed a significant ($P < 0.1$) zygotic SD, and the rest of them were gametic. Among the 97 markers, 79 markers showed maternal allelic SD and 26 showed paternal allelic SD (Supplementary file 3). This result suggests that SD can be related to gametic selection, which plays a major role in segregation distortion (Ma et al, 2015). However, there is a possibility that the effect of zygotic selection on segregation distortion might be underestimated due to parental markers which are not informative in F1 population for zygotic SD tests (Li et al. 2011).

Table 4.35. Number of segregation and skewed markers in Siirt, Pa-18, Consensus map.

Segregation type	Siirt		Pa-18		Consensus	
	Number of mapped markers	Number of skewed markers	Number of mapped markers	Number of skewed markers	Number of mapped markers	Number of skewed markers
abxcd	160	47	160	47	158	46
efxeg	38	11	38	11	38	11
hkxhk	5	1	5	1	5	1
nnxnp	-	-	82	9	82	8
lmxll	103	34	-	-	103	34
Total	306	93	285	68	386	100
% Distortion	-	30.4	-	23.9	-	25.9

When three or more markers with genotypic SD were clustered together, the group of markers were considered as a candidate SDR if the markers in the group showed similar allelic and/or genotypic distortion patterns and had similar allelic and zygotic SD tests (Li et al, 2011). In this study six candidate SDRs were identified and located on LG2, LG5, LG6, LG8, LG9, and LG15 (Figure 1; Supplementary file 3). Inter-specific population maps in this study and intra-specific population constructed by Khodaeiaminjan et al (2017b) revealed that only segregation distortion regions (SDRs) on the LG2, LG6 and LG8 were overlapped. These results showed that the skewed markers in the two mapping populations were different (Figure. 4.26; Supplementary file 3).

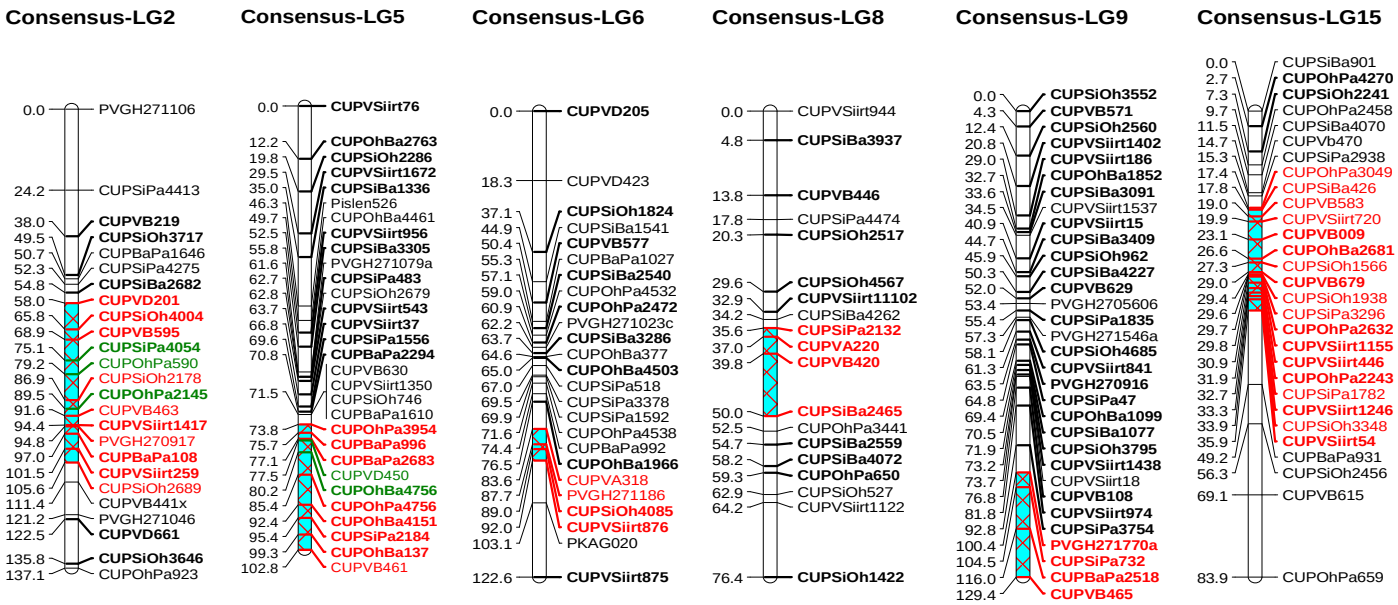


Figure 4.26. Genetic linkage maps of intra- specific population of *Pistacia* populations based on SSR markers. Red regions of linkage groups considered to be candidate segregation distortion regions (SDR). Bold letters indicate the common-segregation SSR loci. Markers in red represented segregation distortion at P<0.1. Markers in green on F1 linkage maps did not show distortion in SDRs .

4.7. Mapping of Sex-Linked Markers in 'Siirt x Pa-18' F1 Population

In this study, the sex-linked SNP and SCAR markers in the literature were also mapped in 'Siirt x monoecious Pa-18' F1 population. The segregation types of the 16 sex-linked markers developed by Kafkas et al (2015) and Khodaiaminjan et al. (2017a) were as follows: 4 markers which were heterozygous in both parents (1 'efxeg' and 3 'hkxhk') and 12 SNPs had 'lmxll' segregation which were only heterozygous in female parent (Figure 4.27)

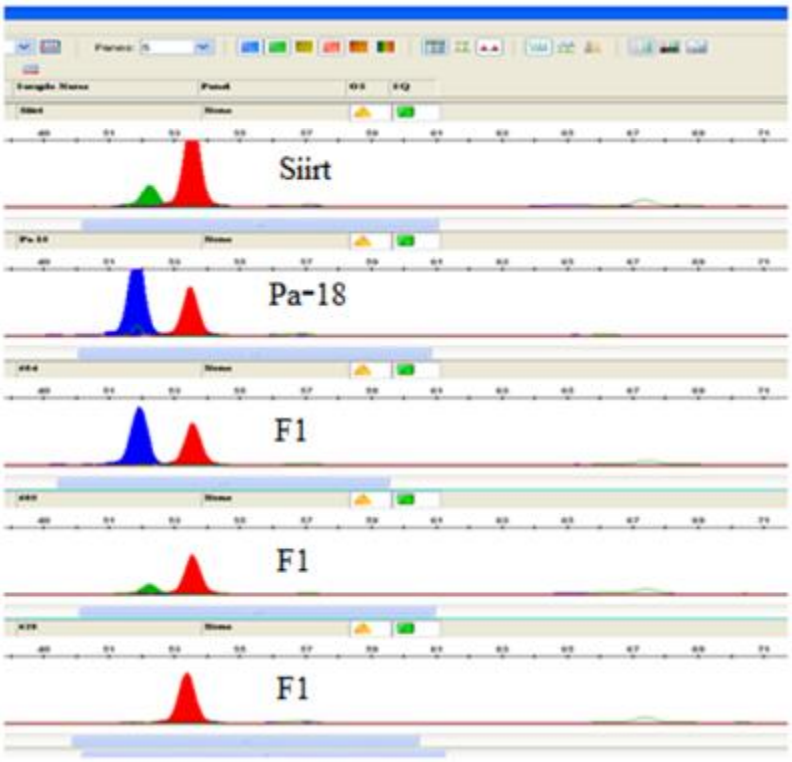


Figure 4.27. The SNP marker (SNP-165792) were heterozygous in both parents. The green peak represents adenine nucleotide. The red and blue peaks represent guanine and thymine nucleotides, respectively.

The SCAR marker (PVF1) had amplification product in female Siirt cultivar and it was absent in monoecious Pa-18 genotype (Figure 4.28). The

amplification of the female-specific band was observed only in 36 F1 progenies when it was tested in 126 F1 progenies.

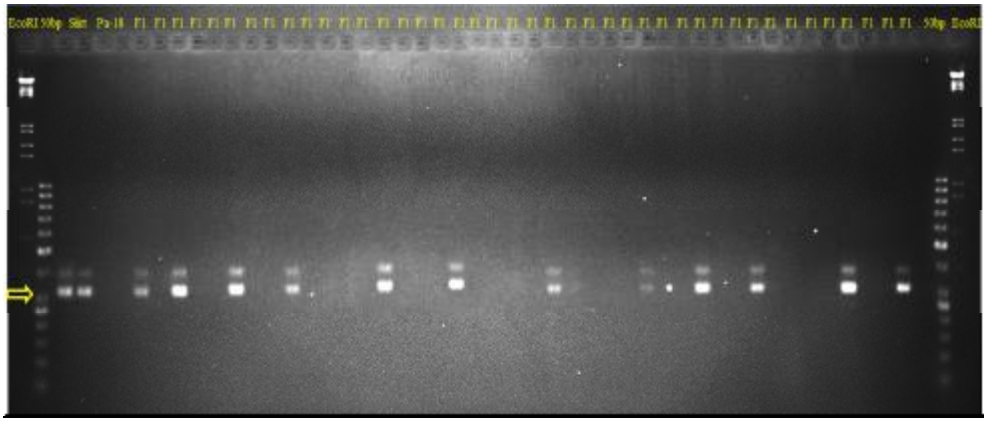


Figure 4.28. Amplification of female-specific PVF1-SCAR marker in F1 progenies and in the parents.

After adding the sex linked markers to the LG1, the consensus LG1 map had 45 markers with a total length of 47.5 cM. The numbers of abxcd, abxc0, efxeg, hkxhk, lmxll lmx00, nnxnp and 00xnp segregations in the consensus map were 5, 2 3, 3, 20, 5, 6 and 1 respectively. A total of 13 common markers were mapped in the LG1 consensus map. Among mapped 45 SSR markers in the consensus map, two markers had distorted segregation. The average distance between SSR markers was 1.05 cM and the average marker density was 0.94. There were no gaps larger than 10 cM in the consensus LG1 map (Figure 4.28 and Table 4.36). The consensus LG1 map of Khodaeiaminjan et al, (2017a) constructed by 42 markers of which 16 markers were sex-linked SNPs. The length of LG1 with SSR, SNP and SCAR markers in Khodaeiaminjan et al (2017a) study was 73.4 cM which was longer than LG1 in this study. The average distance between SSR markers was better in this study (1.05 cM) than in the study by Khodaeiaminjan et al (2017a; 1.74 cM).

In ‘Siirt’ cultivar, the LG1 map was 44.2 cM length with 39 markers. The average distance between the markers became 1.13 cM and the average marker density was 0.88 markers per cM (Supplementary files 4). LG1 of ‘Pa-18’ monoecious genotype had 21 markers, and the total LG length was 26.0 cM. The average distance between the markers was 0.80 cM and the average marker density was 1.23 (Figure 4.29 and Supplementary files 4).

In this study, 12 SNP markers had lmxll segregtions, while 4 markers had the efxeg and hkxhk segregation. One out of four markers had the efxeg segregation which was located in top of LG1, whereas the others had the hkxhk segregation. The lmxll segregated SNP markers were together in the map, while the markers that are heterozygote in both parents are located far away from sex locus. However, those four SNP markers together with 12 SNPs were located in the same locus when Khodaeiamminjan et al. (2017a) mapped them using Siirt (female) x Bağyolu (male) intra-specific F1 population. In addition, PVF1 marker was mapped in the top of LG1 (0 cM) in this study, while it was mapped close to sex locus (2.3 cM far) in Khodaeiaminjan et al (2017a) study. Due to juvenility stage of F1 progenies, it was impossible to phenotype their sex. Since, we just currently know only one progeny sex in the population.

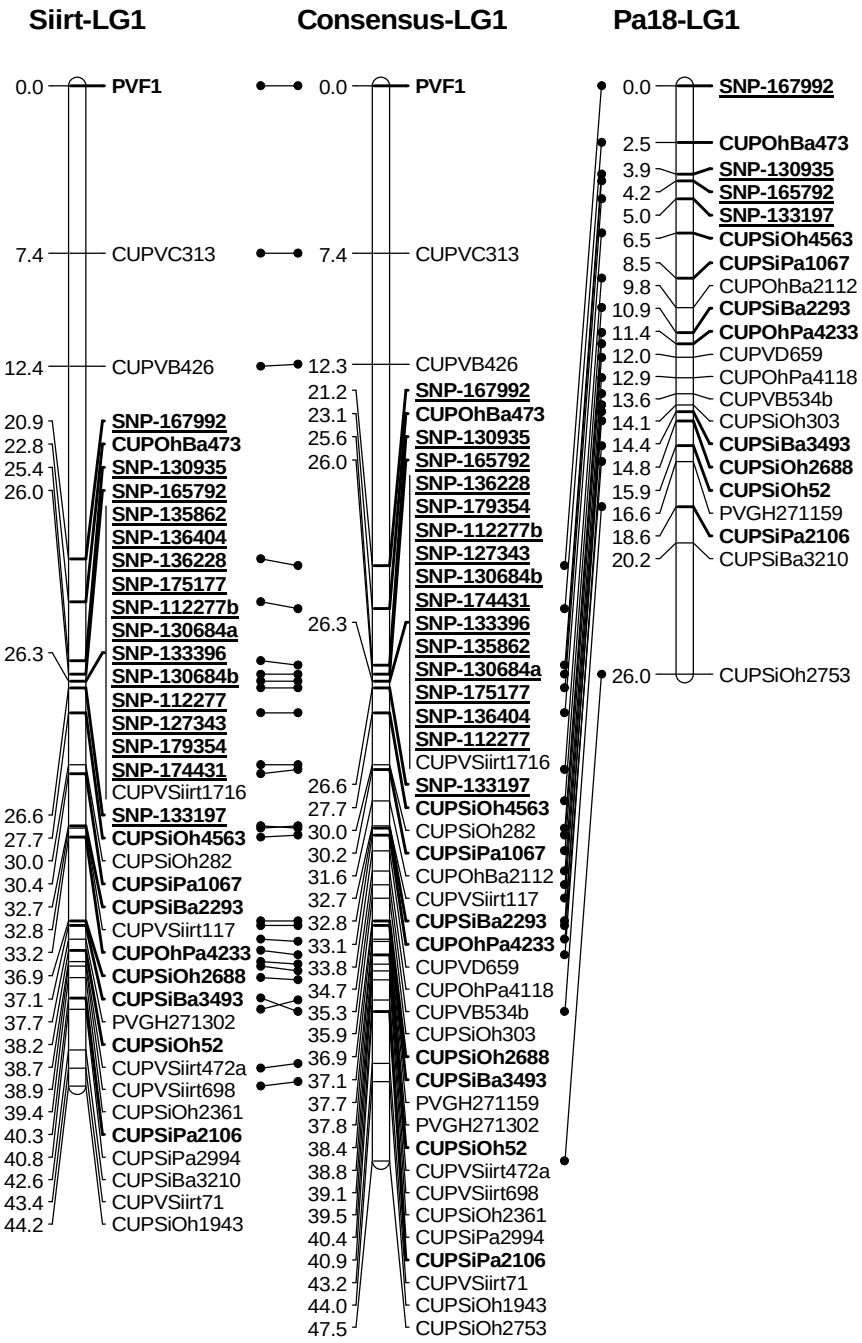


Figure 4.29. LG1 of parental and consensus maps in 'Siirt x Pa-18' F1 population with SNP and SCAR sex-associated markers. The sex-associated SCAR and SNP markers are given in bold.

Table 4.36. Locus name, positions (cM), segregation type, chi-square values (X^2) and significant levels of distorted SSR, SCAR and SNP loci in consensus LG1 map of 'Siirt x Pa-18' F1 population.

No	Locus Name	Position	Classification	X^2	Significance
1	PVF1	0.0	lmxll	24.89	*******
2	CUPVC313	7.4	lmxll	0.07	-
3	CUPVB426	12.3	lmxll	0.03	-
4	SNP-167992	21.2	hkxhk	0.26	-
5	CUPOhBa473	23.1	abxcd	4.18	-
6	SNP-130935	25.6	hkxhk	0.26	-
7	SNP-165792	26.0	efxeg	0.54	-
8	SNP-136228	26.3	lmxll	0.51	-
9	SNP-179354	26.3	lmxll	0.51	-
10	SNP-112277b	26.3	lmxll	0.51	-
11	SNP-127343	26.3	lmxll	0.51	-
12	SNP-130684b	26.3	lmxll	0.51	-
13	SNP-174431	26.3	lmxll	0.51	-
14	SNP-133396	26.3	lmxll	0.51	-
15	SNP-135862	26.3	lmxll	0.51	-
16	SNP-130684a	26.3	lmxll	0.51	-
17	SNP-175177	26.3	lmxll	0.51	-
18	SNP-136404	26.3	lmxll	0.51	-
19	SNP-112277	26.3	lmxll	0.51	-
20	CUPVSiirt1716	26.3	lmx00	0.40	-
21	SNP-133197	26.6	hkxhk	0.52	-
22	CUPSiOh4563	27.7	abxc0	0.88	-
23	CUPSiOh282	30.0	lmx00	0.79	-
24	CUPSiPa1067	30.2	abxcd	4.73	-
25	CUPOhBa2112	31.6	nnxnp	0.20	-
26	CUPVSiirt117	32.7	lmxll	0.10	-
27	CUPSiBa2293	32.8	efxeg	1.10	-

Table 4.35. Continues

28	CUPOhPa4233	33.1	abxcd	0.80	-
29	CUPVD659	33.8	nnxnp	0.39	-
30	CUPOhPa4118	34.7	nnxnp	0.03	-
31	CUPVB534b	35.3	nnxnp	0.01	-
32	CUPSiOh303	35.9	nnxnp	0.21	-
33	CUPSiOh2688	36.9	abxc0	0.19	-
34	CUPSiBa3493	37.1	abxcd	0.18	-
35	PVGH271159	37.7	nnxnp	0.07	-
36	PVGH271302	37.8	lmxll	0.13	-
37	CUPSiOh52	38.4	efxeg	0.41	-
38	CUPVSiirt472a	38.8	lmx00	0.00	-
39	CUPVSiirt698	39.1	lmxll	0.01	-
40	CUPSiOh2361	39.5	lmx00	0.03	-
41	CUPSiPa2994	40.4	lmxll	0.40	-
42	CUPSiPa2106	40.9	abxcd	0.35	-
43	CUPVSiirt71	43.2	lmx00	0.04	-
44	CUPSiOh1943	44.0	lmxll	0.03	-
45	CUPSiOh2753	47.5	00xnp	4.30	**

(**) $p<0.01$, (*******) $p<0.00005$.

In the current study, the sex-linked markers developed by different authors (Yakubov et al, 2005 and Kafkas et al, 2015; Khodaeiamminjan et al (2017a) were mapped in LG1 between 0.0 cM and 26.5 cM. Twelve sex-linked SNP markers were located at the position of 26.3 cM, which is supposed to be the sex locus. The SSR marker CUPVSiirt1716 was mapped in the sex locus as well. CUPVSiirt1716 marker had the lmxll segregation type with two alleles (140-160 bp) in female parent and null allele in male parents. CUPVSiirt1716 marker would be a good SSR marker for sex determination. To prove this hypotheses we tested it in 12 female and 12 male *P. vera* cultivars. The female cultivars had both alleles

(heterozygote), while 10 of 12 male cultivars were homozygote and two were heterozygote.

The SNP-167992 and SNP-130935 markers had hkxhk segregation and were mapped in 21.2 and 25.6 cM respectivly. The SNP-165792 marker had efxeg segregation type and was mapped in 26.0 cM and it was 0.3 cM away from SNP-130935. Due to juvenility stage of F1 progenies, it was impossible to phenotype sex of F1 progenies.

4.8. QTL Analysis of Leaf and Shoot Traits In Pistachio

4.8.1. Correlations Among The Traits

Measurements of leaf length (LL), leaf width (LW), number of leaflets (NLL), young shoot color (YSC), and leaf color (LC) of F1 individuals derived from a cross between *P. vera* 'Siirt' cultivar and monoecious *P. atlantica* 'Pa-18' genotype which were done during two consecutive years (2015; 100 F1 and 2016; 100 F1). There was significant variations in leaf length (LL), leaf width (LW), number of leaflets (NLL), young shoot color (YSC), and leaf color (LC) within the F1 population. Leaf length and leaf width were significantly correlated ($r \geq 0.8$) in both 2015 and 2016 gowing seasons. There were no significant correlations between the other traits. (Table 4.37 and 38).

Table 4.37. The correlations between leaf length (LL), leaf width (LW), number of leaflet (NLL), young shoot color (YSC), and leaf color (LC) in 2015 growing season.

The corelation of leaf length (LL), leaf width (LW), number of leaflet (NLL), young shoot color (YSC), and leaf color (LC) in **2015 growing seasone**					
	Leaf length	**Leaf width**	**Number of**	**Young shoot**	**Leaf color**
Leaf length	1.00				
Leaf width	**0.87**	1.00			
Number of	-0.23	-0.34	1.00		
Young shoot	-0.19	-0.23	-0.07	1.00	
Leaf color	0.11	0.11	0.03	-0.06	1.00

Table 4.38. The correlations between leaf length (LL), leaf width (LW), number of leaflet (NLL), young shoot color (YSC), and leaf color (LC) in 2016 growing season.

The corelation of leaf length (LL), leaf width (LW), number of leaflet (NLL), young shoot color (YSC), and leaf color (LC) in 2016 growing seasone					
	Leaf	**Leaf width**	**Number of**	**Young shoot**	**Leaf color**
Leaf length	1.00				
Leaf width	**0.81**	1.00			
Number of	-0.04	0.10	1.00		
Young shoot	-0.34	-0.42	-0.15	1.00	
Leaf color	-0.04	-0.21	-0.05	0.33	1.00

4.8.2. Frequency Distribution of The Morphological Traits

The frequency distribution of leaf length (LL), leaf width (LW), number of leaflets (NLL) and young shoot color (YSC) in the progenies from the 'Siirt × Pa-18' F1 population were done during two consecutive years.

4.8.2.1. Leaf length (LL)

The leaf length of the parents and F1 population were measured. The two parents Siirt and monocious *P. atlantica* 'Pa-18' differed in leaf length. The leaf length of Siirt cultivar was 16.8 and 15.9 cm in 2015 and 2016, respectively, whereas it was 13.6 and 13.2 cm in 'Pa-18' genotype. The ranges of leaf length (LL) values varied from 7.1 to 14.6 cm in year 2015, and 6.2 to 14.5 cm in year 2016. The average leaf length for two years; 2015 and 2016 ranged from 11.6 to 10.9 cm respectively (Table 4.36). The frequency distribution of two years of LL values gave a good fit to normal distribution (as shown in Table 4.39 and Figure.4.30).

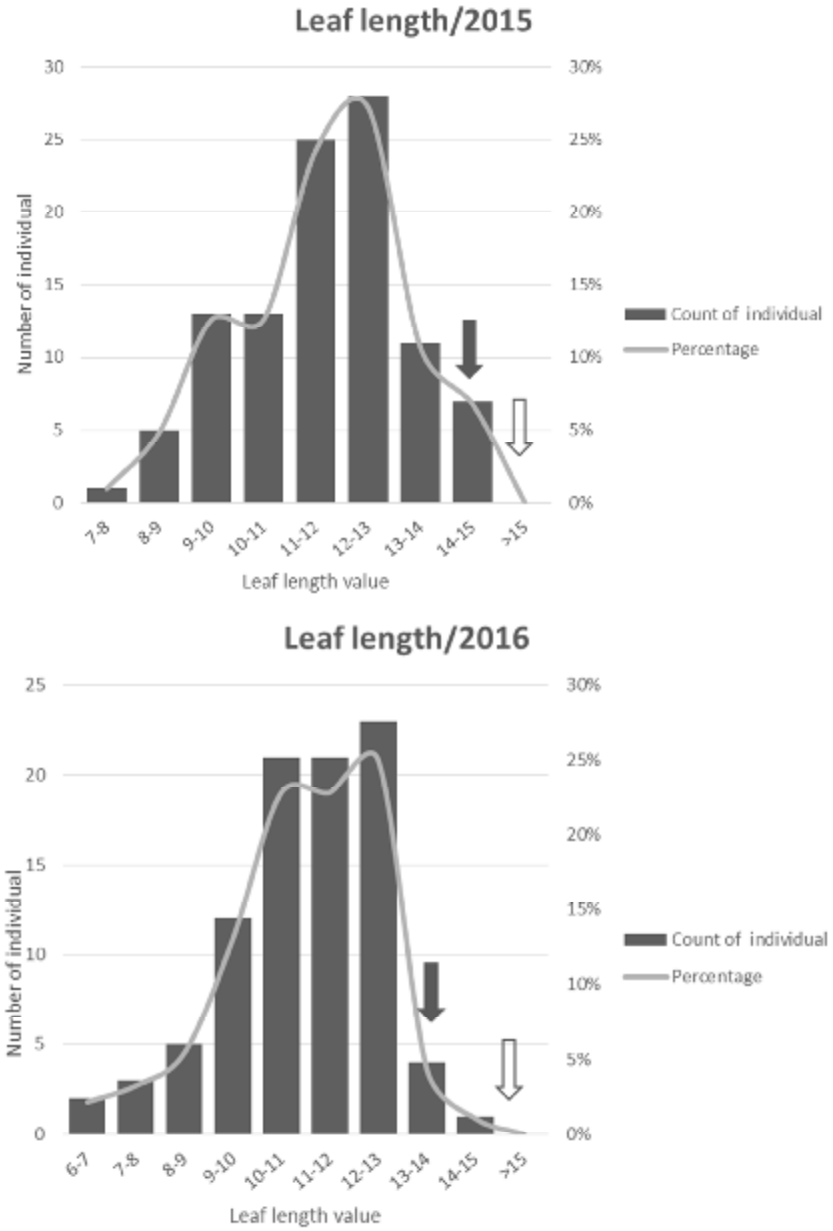


Figure 4.30. Frequency distributions of leaf length (LL). Phenotypic data were collected in 2015 and 2016. The 'Siirt' values are indicated white arrow and 'Pa-18' with black arrow.

Table 4.39. Mean values, maximum and minimum values, standard deviation of all traits evaluated in 2015 and 2016.

Trait	Year	F1 Progenies				Parents	
		Mean	Max	Min	SD	Siirt	Pa-18
LL	2015	11.6	14.6	7.1	1.59	16.8	13.6
	2016	10.9	14.5	6.2	1.57	15.9	13.2
LW	2015	8.8	11.5	5.6	1.21	15.3	9.9
	2016	8.3	11.9	5.1	1.24	14.8	9.6
NLL	2015	2.5	3	2	0.34	1.4	2.3
	2016	2.7	3	2	0.34	1.5	2.5
YSC*	2015	2.0	3	1	0.58	1*	3**
	2016	2.3	3	1	0.75	1*	3**
LC	2015	43.2	49.6	36.1	2.35	50.8	36.5
	2016	46.3	53.0	38.7	2.74	52.2	38.4

*very light brown color; **represents brown color

4.8.2.2. Leaf width (LW)

The measurements of leaf width of parents and F1 population were performed. The leaf width of Siirt cultivar was 15.3 and 14.8 cm in 2015 and 2016 respectively, while the leaf width of 'Pa-18' genotype was 9.9 and 9.6 cm. The ranges of leaf width (LW) values varied from 5.6 to 11.5 cm in 2015 and it was 5.1 to 11.9 cm in 2016. The frequency distribution of leaf width was a normal distribution in both years (Table 4.38 and Figure4.31).

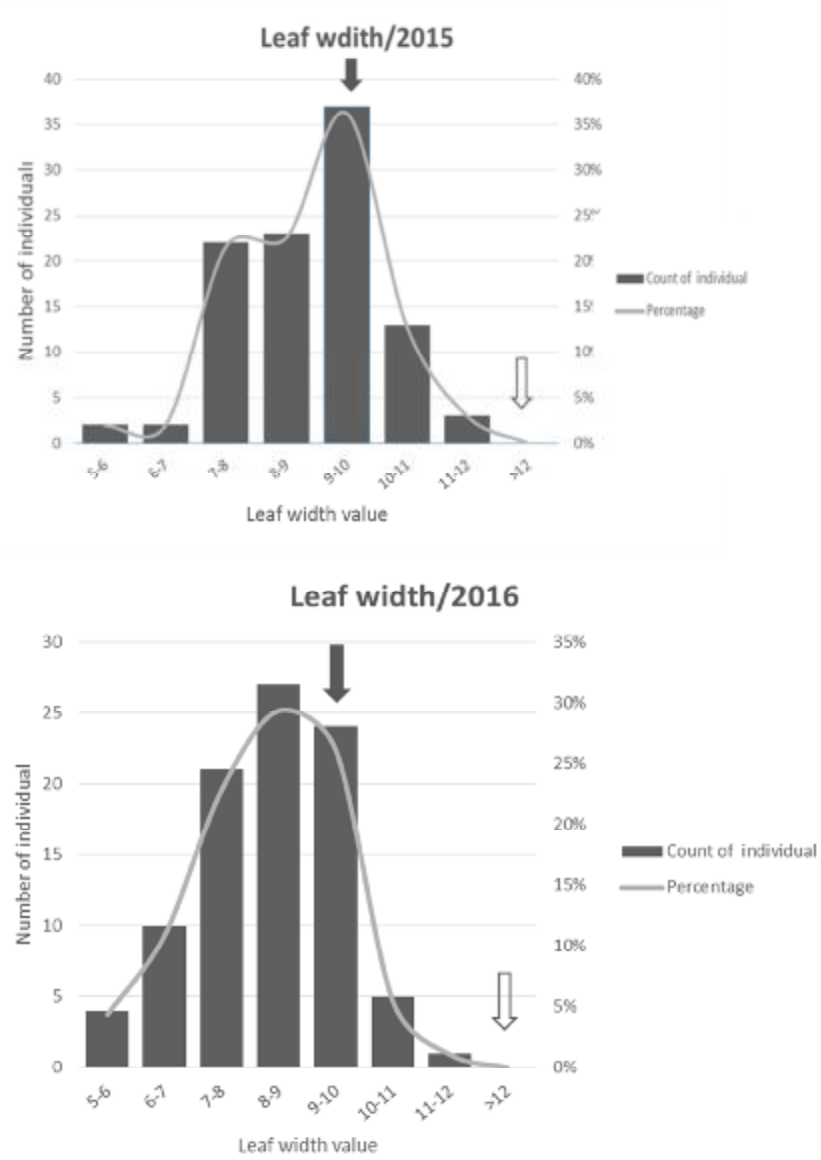


Figure 4.31. Frequency distributions of leaf width (LW). Phenotypic data were collected in 2015 and 2016. The 'Siirt' values are indicated white arrow and 'Pa-18' with black arrow.

4.7.2.3. Number of Leaflets (NLL)

The result of leaflet count in the leaves of the parents (Siirt and monocious *P. atlantica* 'Pa-18') and F1 population were different. The average number of leaflets during two years ranged from 2.5 to 2.7 respectively. The number of leaflets of Siirt cultivar was 1.4 and 1.5 in two consecutive years (2015 and 2016), whereas the number of leaflets of 'Pa-18' genotype were 2.3 and 2.5. The ranges of number of leaflets (NLL) values varied from 2 to 3 in both two years; 2015 and 2016 (Table 4.36). The distribution of two years of NLL values was assessed the multimodal distribution which had more than a few peaks close together. The number of modes corresponds to the number of effective competitors. Their separation is a consequence of average distances among individuals. The multimodality may indicate that the sample is not homogenous (Table 4.38 and Figure 4.32).

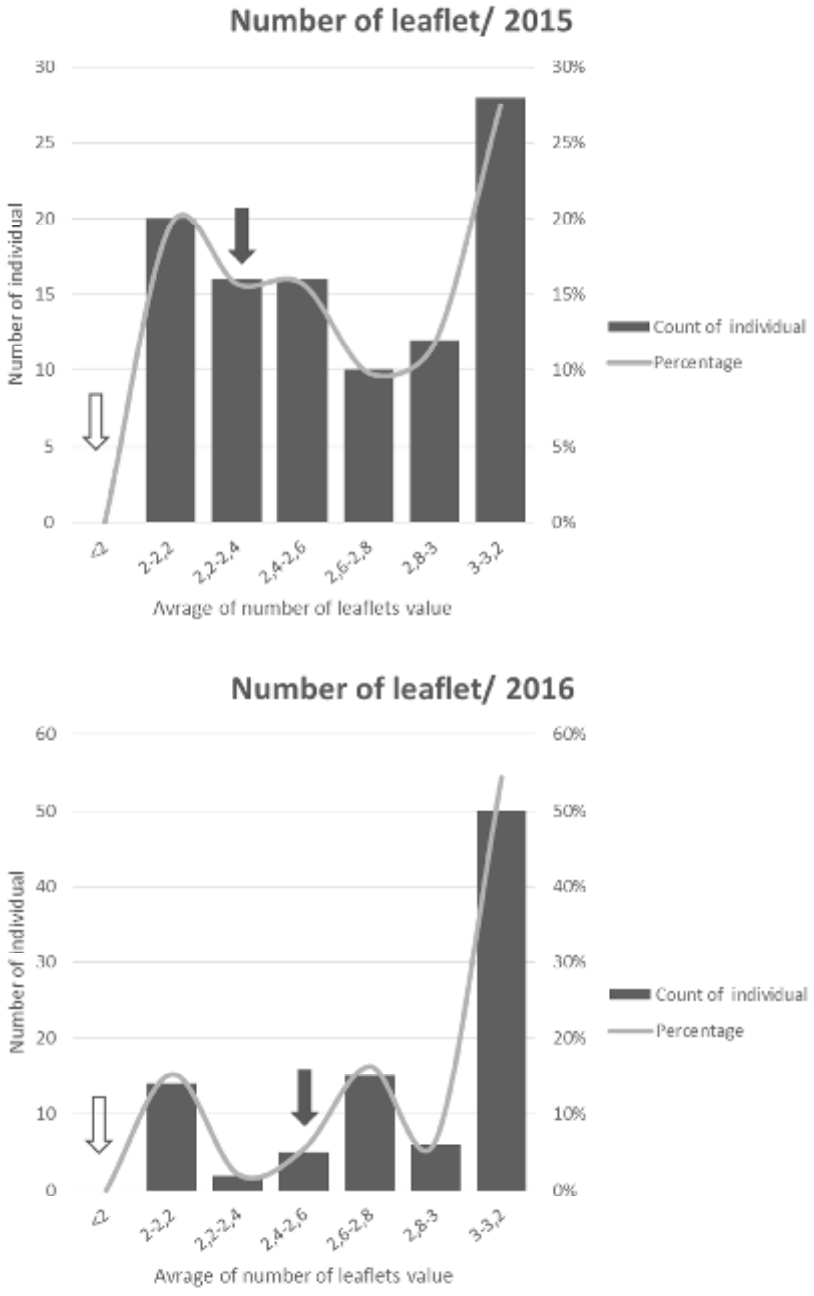


Figure 4.32. Frequency distributions of number of leaflets (NLL). Phenotypic data were collected in 2015 and 2016. The 'Siirt' values are indicated white arrow and 'Pa-18' with black arrow.

4.8.2.4. Young Shoot Color (YSC)

Young shoot color values varied from very light brown (1), light brown (2) and brown (3) in each year. According to the observations, the young shoot color of two parents Siirt and monoecious *P. atlantica* 'Pa-18' were different. The young shoot color of Siirt cultivar was very light brown, while 'Pa-18' genotype was brown (Table 4.36). The distribution of two years of YSC values was assessed as normal distribution because more than 70% of the progenies had above or below the parental values. (Table 4.38 and Figure 4.33).

4.8.2.5. Leaf Color (LC)

The leaf color of the parents and F1 progenies were also measured (Figure 4.33). The leaf color of Siirt cultivar was calculated as 50.8 in 2015 and 52.2 in 2016 with HunterLab instrument, whereas the leaf color of 'Pa-18' genotype was 36.5 in 2015 and 38.4 in 2016.

The ranges of leaf color (LC) values varied from 43.2 to 49.6 in 2015, and 46.3 to 53.0 in 2016. The averages of leaf color in 2015 and 2016 were 36.1 to 38.7 respectively (Table 4.38). The distribution of data in 2015 of NLL values was assessed the bimodal distribution which had two peaks and the distribution of data in 2016 was indicated as multimodal distribution (Table 4.38 and Figure 4.34).

Among the assessment of five traits, leaf length (LL), leaf width (LW) and young shoot color (YSC) showed a normal distribution in two consecutive years, while the number of leaflets (NLL) and leaf color (LC) showed multimodal distribution in 'Siirt x Pa-18' F1 population.



Figure 4.33. Frequency distributions of young shoot color (YSC). Phenotypic data were collected in 2015 and 2016. The 'Siirt' values are indicated white arrow and 'Pa-18' with black arrow

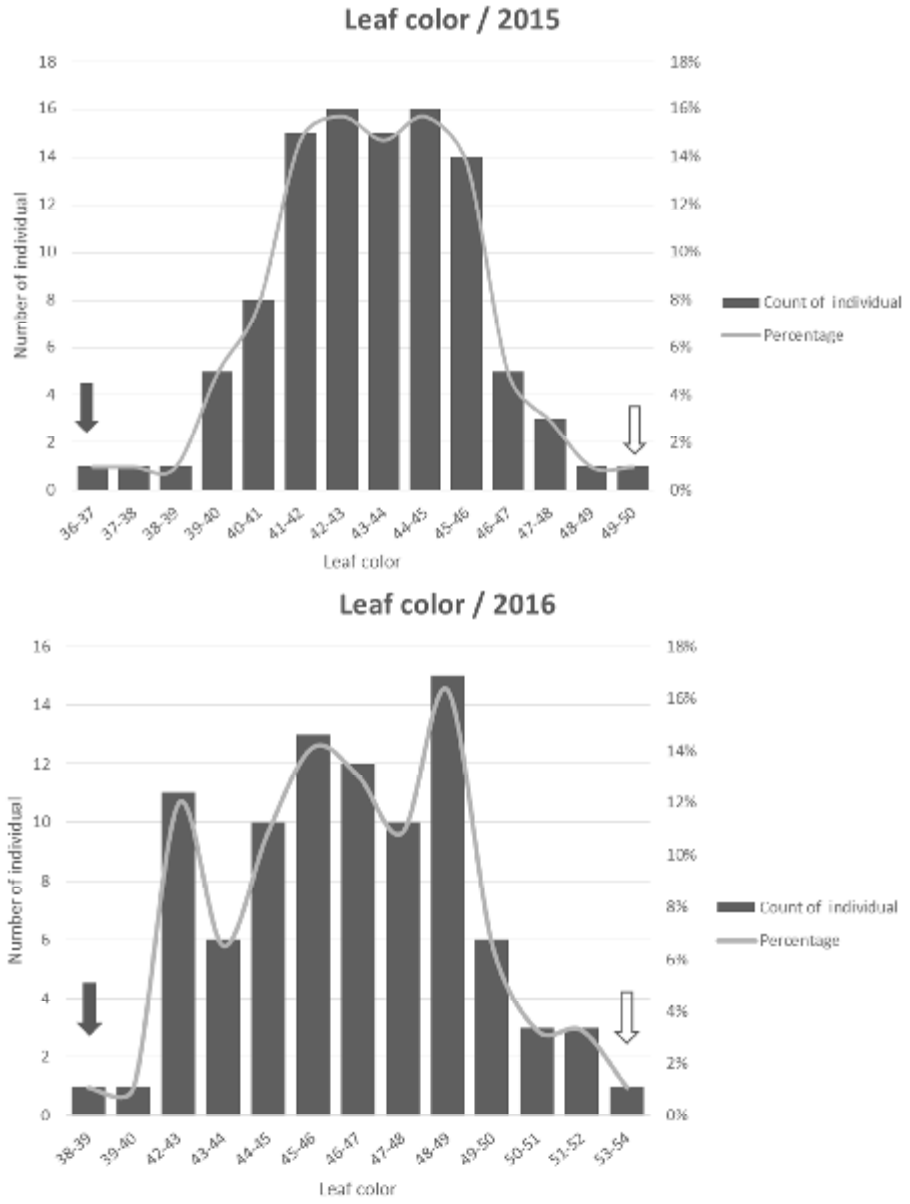


Figure 4.34. Frequency distributions of leaf color (LC). Phenotypic data were collected in 2015 and 2016. The 'Siirt' values are indicated white arrow and 'Pa-18' with black arrow.

4.9. QTL Identification for Leaf and Shoot Traits In Pistachio

The parental maps were used in the QTL analysis. From a total of five traits, 17 QTLs were detected in the parental maps. The 'Siirt' map had nine QTLs, and the 'Pa-18' map had 8 QTLs detected in both consecutive years.

4.9.1. Leaf Length (LL)

In the 'Siirt' × 'Pa-18' F1 population, leaf length varied from 7 to 15 cm in 2015, and from 6 to 15 in 2016. Two significant QTLs for LL were mapped into LG2 and LG4. The highest LOD was scored in 2015 with a percentage of phenotypic variation explained of 10.6% in female, and 15.2% in male dataset. The higher LOD of QTLs for leaf length was found at the top of LG2 of female, and the middle of male maps (Table 4.39, Figure 4.34). In female, LG2 map is shorter and some of markers which were located in the top of LG2 in male map were not available in female map. The difference in map position can be explained by having different linkage length in male and female maps. (Table 4.39, Figure 4.35). The closest marker in LG2 was 'CUPSiBa2682' (15.7 cM) in both maps and KW tests were significant at $p < 0.01$ at the highest LOD with a 10.6% and 15.2% percentage of explanation phenotypic variation in Siirt and Pa-18 map respectively. The closest marker in LG4 was 'CUPBaPa667' (55.9cM) SSR marker in female map and 'CUPSiPa124' (34.7 cM) in male map.

4.9.2. Leaf Width (LW)

In the 'Siirt' × 'Pa-18' F1 population, LW varied from 5 and 12 cm in 2015, and 2016. Three significant QTLs for LW were mapped in LG2, LG4 and LG12. The highest LOD was scored in 2015 with a phenotypic variation explained of 13.3% in female, and 17% in male dataset. The higher LOD of QTLs for leaf width was found at the bottom of LG9 in both maps (Table 4.39, Figure 4.35). The closest marker in LG9 was 'CUPSiBa2682' (15.7 cM) in both maps with KW $p<0.005$. The closest marker in LG4 was 'CUPBaPa667' (55.9 cM) in female map,

and ‘CUPSiPa124’ (34.7 cM) in male map. The closest marker in LG12 was ‘CUPVD414’ (20 cM) in female map.

Table 4.40. Summary of the QTLs in the 'Siirt' × 'Pa-18' population.

Trait name	Year	Siirt Map	CM	LOD	% Expl.[a]	Marker	Kruskal-Wallis test significance	Pa-18 Map	CM	LOD	% Expl.	Marker	Kruskal-Wallis test significance
Leaf Length	2015	S2	10-40	2.3	10.6	CUPSiBa2682 (15.7cM)	0.01	P2	50-80	3.11	15.2	CUPSiBa2682(54.8cM)	0.01
Leaf Length	2016	S2	10-20	1.5	7.7	CUPSiBa2682 (15.7cM)	0.05	P2	50-65	1.87	11.1	CUPSiBa2682(54.8cM)	0.05
Leaf Length	2015	S4	50-63	2.03	9	CUPBaPa667 (55,6cM)	0.01	P4	25.50	3.02	15.9	CUPSiPa224 (34,7cM)	0.001
Leaf Length	2016	S4	52-65	1.5	8.5	CUPBaPa667 (55,6cM)	0.05	P4	30.52	2	10.7	CUPSiPa224 (34,8cM)	0.01
Leaf Width	2015	S2	15-40	3.1*	13.3	CUPSiBa2682 (15,75cM)	0.005	P2	52-85	3.6*	17	CUPSiBa2682(54.8cM)	0.005
Leaf Width	2016	S2	0-20	2.5	14.3	CUPSiBa2682 (15.7cM)	0.005	P2	52-65	3.47*	19	CUPSiBa2682(54.8cM)	0.01
Leaf Width	2015	S4	40-60	2.5	11.1	CUPBaPa667 (55,6cM)	0.01	P4	20-50	2.99	17.2	CUPSiPa224 (34,8cM)	0.001
Leaf Width	2016	S4	55-65	2.1	10.6	CUPBaPa667 (55,6cM)	0.01	P4	20-55	2.25	12.3	CUPSiPa224 (34,8cM)	0.005
Leaf Width	2015	S12	5-30	2.17	48.9	CUPVD414 (20cM)	0.005	-	-	-	-	-	-
Leaf Width	2016	S12	10-30	3.15*[a]	19.5	CUPVD414 (20cM)	0.01	-	-	-	-	-	-
Number of leaflets	2015	S7	40-55	4.67**	23.6	CUPVSiirt1117 (47,3cM)	0.0001	P7	40-65	4.9**	25.2	CUPSiPa2473 (43,7cM)	0.005
Number of leaflets	2016	S7	50-60	1.5	9.9	CUPSiOh4114 (55,5 cM)	0.05	P7	55-65	3.22*	18	CUPOhPa4736 (66,3 cM)	0.01
Young shoot color	2015	S9	65-86	2.31	10.3	CUPOhBa1007 (69.3cM)	0.01	P9	70-88	2.31	10.3	CUPOhBa1007 (69.3cM)	0.01
Young shoot color	2016	S9	71-115	2.26	11.4	CUPSiOh3795 (72cM)	0.05	P9	80-120	2.26	11.4	CUPSiOh3795 (72cM)	0.05
Color value	2015	S7	15-30	3.48*	14.9	CUPOhPa819 (19,8cM)	0.01	P7	15-30	3.48*	15	CUPOhPa819 (16,9cM)	0.0001
Color value	2016	S7	18-30	3.48*	19.6	CUPOhPa819 (19,8cM)	0.05	P7	15-30	2.06	10	CUPOhPa819 (19,8cM)	0.05
Color value	2015	S9	3-30	2.06	11	CUPVB571 (4,2cM)	0.01	P9	3-30	2.31	21.1	CUPVB571 (4,3cM)	0.01
Color value	2016	S9	0-15	2.28	11	CUPVB571 (4,2cM)	0.01	P9	0-15	2.28	11	CUPVB571 (4,3cM)	0.01

a Percentage of explanation phenotypic variation

b Genome wide significant levels: *P < 0.05, **P < 0.01

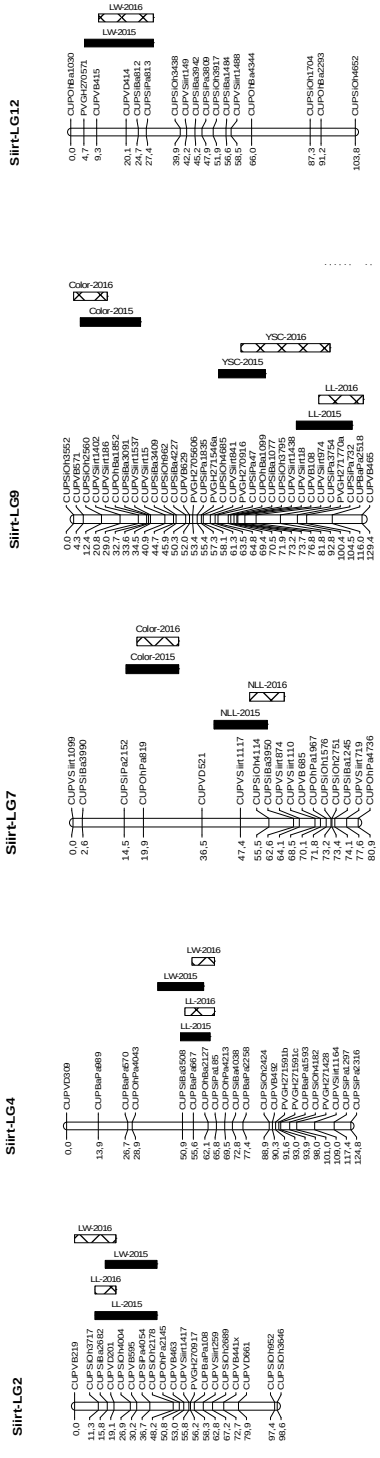


Figure 4.35. Parental (Siirt' and 'Pa-18') linkage maps with positions of QTLs related to the phenotypic traits. Having the significant QTLs in the five linkage groups (LG) 2, 4, 7, 9 and 12 of 'Siirt' and the four linkage groups (LG) 2, 4, 7 and 91 of 'Pa-18' maps were presented. The black and striped bars on the right side of the LGs indicate the QTLs which are detected in 2015 and in 2016, respectively.

4.9.3. Number of Leaflets (NLL)

Number of leaflets (NLL) varied from 2 to 3 in both years. One significant QTL for NLL was mapped in LG7. The highest LOD was scored in 2015 with 23.6% explained phenotypic variation, and KW test $p<0.0001$ in female, and 25.2% in male with KW test $p<0.005$. (Table 4.39, Figure 4.35). The closest markers in 2015 and 2016 in LG7 were CUPVSiirt1117 (47.3 cM) and CUPSiOh4114 (55.5 cM) in female map, and CUPSiPa1473 (43.7 cM) and CUPOhPa4736 (66.3 cM) in male map respectively.

4.9.4. Young Shoot Color (YSC)

In this study, observation of young shoot color (YSC) was done as (1) very light brown, (2) light brown and (3) brown. One significant QTL for YSC was mapped in LG9. The explained phenotypic variation of YSC was scored as the highest LOD in both female and male in 2015 with 10.3% of dataset. The higher LOD were significant at $p < 0.01$ in 2015, and $p<0.05$ in 2016 in both female and male maps. The QTLs for young shoot color was found at the bottom of LG9 in both maps (Table 4.39, Figure 4.35). The closest markers in 2015 and 2016 in LG9 were CUPOhBa1077 (69.3 cM) and CUPSiOh3795 (72.0 cM) respectively, in both maps.

4.9.5. Leaf Color (LC)

In the ‘Siirt’ × ‘Pa-18’ F1 population, LC assessed with HunterLab instrument and ranged from 36 to 53 in 2015 and 38 to 54 in 2016. Two significant QTLs for LC were mapped in LG7 and in LG9. The highest LOD was scored in 2015. The higher LOD of QTLs for leaf color was found at the top of LG7 in both maps with a percentage of phenotypic variation explained of 14.9% at $p<0.01$ in 2015, and at $p<0.05$ in 2016 in female map, and 15% ($p<0.01$) in 2015 at $P<0.05$ in 2016 in male dataset (Table 4.39, Figure 4.35). The closest marker in LG7 was ‘CUPOhPa819’ (19.8 cM) in both maps.

This is the first study on QTL analysis in the genus *Pistacia*. In this study, some of phenotypic traits were detected in more than one linkage group. The QTLs of leaf length and leaf width were detected in LG2, LG4 and LG12 in 'Siirt' map and also were located on LG2 and LG4 in 'Pa-18' map. The QTLs for the number of leaflets were detected in LG7 in both maps. The QTL of young shoot color were detected in LG9 in both maps, and the QTLs for leaf color were detected in LG7 and LG9 in both parental maps. The occurrence of multiple QTLs at different positions can be interpreted as complicated genes which control leaf information.

There are several studies reporting QTLs for leaf and shoot traits in plants (Mian et al, 1998; Dunemann et al, 1999; Jiao et al, 2010; Baker et al, 2015; Tan et al, 2016). However, there was no report on QTL analysis of leaf charecters in pistachio. In the last several decades, many articles were published on QTL analysis in tree plants. However, this is the first QTL analysis report in the genus *Pistacia*. In this study, leaf charecters tended to be a complex trait in *Pistacia*. It was also one of the complex traits in the other plant species such as tomato (Frary et al, 2004) and grapevine (Welter et al, 2007).

The main crossing female *P. vera* with monoecious *P. atlantica* in this study was (1) to breed monoecious cultivated pistachio and (2) to study sex inheritance and mechanism in *Pistacia* (Kafkas, 2003). However, the progenies are still at juvenility stage and several plants have died per year since they were planted. In addition, the growing habits of the progenies are weaker than other F1 populations in the breeding program such as 'female *P. vera* x male *P. vera*' or 'female *P. vera* x male *P. atlantica*'.

Inter-specific hybridization causing a high frequency of complementary alleles can be great potential for generating novel phenotypes in population (de Vicente and Tanksley, 1993). In the breeding programs, the potential use of molecular markers such as SSRs, which are linked to QTLs, depends on the magnitude of the QTL effects (Dunemann et al, 2009). The detected QTLs for leaf characters were compared to map position in this study. Consequently, twenty-

eight SSRs were reported for leaf characters in both parental maps of *Pistacia* in this study.

5. CONCLUSIONS

In this study an approximately 40× Illumina data for the genome survey in *P. vera* were obtained to have knowledge about the structure of the pistachio genome. The assembled data were also used to search for SSRs in the pistachio genome, to develop novel SSR markers, and to study genetic diversity in six *Pistacia* species. K-mer analysis indicated that the pistachio genome is highly heterozygous and also is about 600 Mb in size. The level of repeats in the pistachio genome was not high, and the GC content was about 37.1%. The SSR search in the assembled genome revealed 59,280 SSRs with a frequency of 8.67 kb. A total of 206 polymorphic SSR loci were developed from 950 SSR loci: 136 had amplifications and 41 were polymorphic in all six *Pistacia* species. In this study the first data on the structure of the pistachio genome was reported and may help to design whole genome sequencing studies in pistachio. Furthermore, current study report novel polymorphic SSR markers for six *Pistacia* species will enable further genetic mapping, genetic diversity, and germplasm characterization studies in the genus.

Four hundred and three SSR markers were mapped in the consensus genetic linkage maps of ‘Siirt’ x ‘Pa-18’ F1 population. The length of total map, consisting 15 linkage groups, was 1,486.1 cM with an average distance of 3.9 cM between adjacent markers. The length of LGs ranged from 41.6 cM (LG1) to 137.1 cM (LG2). The parental genetic linkage maps of ‘Siirt’ and ‘Pa-18’ covers a length of 1,410.4 and 1,362.5 cM in ‘Siirt’ and ‘Pa-18’ respectively, with an average linkage-group length of 94 and 90.8 respectively.

In ‘Siirt’ map, the lengths of linkage groups ranged from 38.7 cM in LG1 to 124.8 cM in LG9. A total of 306 markers were mapped in the maternal parent with an average of 20.4 markers per linkage group, and a mean marker density of 4.6 cM. LG9 had the highest number of markers (32), while LG11 had the lowest

(14). The highest average marker density, 7.7 cM, was in LG6, whereas the lowest density was 1.8 cM in LG1.

In ‘Pa-18’, total of 285 markers were mapped with lengths of linkage groups ranged from 23.4 cM in LG1 to 140.7 cM in LG2. The average number of markers per linkage group was 19 and a mean marker density of 4.8 cM. LG9 had the highest number of markers (28), while LG3, 11 had the lowest (14). The highest average marker density, 6.7 cM, was in LG2, whereas the lowest density was 1.4 cM in LG1.

A total of 203 SSRs were successfully mapped in reference map of ‘Siirt x Pa-18’ F1 population along with15 linkage groups. The final common genetic linkage map was 1,223.03 cM long, consisting 15 linkage groups with an average distance between markers of 6.0 cM. The length of LGs ranged from 23.43 to 131.9 cM.

Among the five traits assessed, leaf length (LL), leaf width (LW) and young shoot color (YSC) showed a normal distribution in both two years. While the number of leaflets (NLF) and leaf color (LC) evaluated multimodal distribution in ‘Siirt x Pa-18’ F1 progeny.

Based on the map, 17 reliable QTLs responsible for leaf length, leaf width, number of leaflets, young shoot color, and leaf color of progenies were detected. The QTLs of leaf length and leaf width were detected in LG2, 4 and 12 to in ‘Siirt’ map and also were located on LG2, LG4 in ‘Pa-18’ map. The QTL of number of leaflets were detected in LG7 in both maps. The QTL of young shoot color in LG9, in both maps and the QTLs of leaf color, were detected in LG7 and LG9 in both maps.

This genetic map is one of the dense genetic maps of *Pistacia* genus. QTLs which were identified in this map can be a source for the morphology determination, and related traiits in this population. Furthermore, using information of QTLs on the map may be applied to be candidate genes for developing functional markers in order to select the marker assist program.

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I was born on Jounary, 04 1985, in Isfahan, Iran. I got my primary and secondary my high school education in Isfahan. I got my BSc degree from Agriculture Department Plant Potology Faculty of Khorasgan University in 2008. In 2013, I finished my master degree in Biotechnology Department of Cukurova University and started my Ph.D degree.

SUPPLEMENTARY FILES

SUPPLEMENTARY FILE-1

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG1 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPVC313	0.0	lmxll	0.07	-
2	CUPVB426	9.1	lmxll	0.03	-
3	CUPOhBa473	17.8	abxcd	4.18	-
4	CUPVSiirt1716	20.6	lmxll	0.40	-
5	CUPSiOh4563	21.5	abxcd	0.88	-
6	CUPSiOh282	23.6	lmxll	0.79	-
7	CUPSiPa1067	23.9	abxcd	4.73	-
8	CUPSiBa2293	26.4	efxeg	1.1	-
9	CUPVSiirt117	26.7	lmxll	0.1	-
10	CUPOhPa4233	27.2	abxcd	0.8	-
11	CUPSiOh2688	30.6	abxcd	0.19	-
12	CUPSiBa3493	30.7	abxcd	0.18	-
13	PVGH271302	31.4	lmxll	0.13	-
14	CUPSiOh52	31.7	efxeg	0.41	-
15	CUPVSiirt472a	32.2	lmxll	0	-
16	CUPVSiirt698	32.5	lmxll	0.01	-
17	CUPSiOh2361	32.8	lmxll	0.03	-
18	CUPSiPa2994	33.9	lmxll	0.4	-
19	CUPSiPa2106	34.5	abxcd	0.35	-
20	CUPSiBa3210	36.7	abxcd	1.85	-
21	CUPSiOh1943	37.4	lmxll	0.03	-
22	CUPVSiirt71	38.7	lmxll	0.04	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG2 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPSiOh3646	0.0	abxcd	4.13	-
2	CUPSiOh952	1.1	abxcd	30.63	*******
3	CUPVD661	18.6	hkxhk	2.58	-
4	CUPVB441x	25.9	lmxll	0.13	-
5	CUPSiOh2689	31.4	lmxll	4.88	**
6	CUPVSiirt259	35.7	abxcd	2	-
7	CUPBaPa108	40.2	efxeg	4.62	-
8	PVGH270917	42.4	lmxll	3.23	*
9	CUPVSiirt1417	42.8	hkxhk	8.34	**
10	CUPVB463	45.6	lmxll	4.65	**
11	CUPOhPa2145	47.8	abxcd	5.72	-
12	CUPSiOh2178	50.3	lmxll	3.9	**
13	CUPSiPa4054	61.9	abxcd	1.74	-
14	CUPVB595	68.4	abxcd	7.71	*
15	CUPSiOh4004	71.7	abxcd	6.63	*
16	CUPVD201	79.5	abxcd	9.65	**
17	CUPSiBa2682	82.8	efxeg	5.72	-
18	CUPSiOh3717	87.3	abxcd	11.46	***
19	CUPVB219	98.6	abxcd	27	*******

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG3 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPVB567	0.0	lmxll	1.14	-
2	CUPSiBa1284	19.6	abxcd	2.39	-
3	CUPOhPa3361	24.2	abxcd	3.4	-
4	CUPSiOh2834	25.6	lmxll	2.35	-
5	CUPBaPa739	27.9	lmxll	3.84	**
6	CUPSiOh390	28.7	abxcd	2.01	-
7	CUPSiBa3907	30.5	lmxll	1.83	-
8	CUPOhBa1934	31.0	lmxll	1.37	-
9	CUPSiBa374	31.4	abxcd	2.43	-
10	CUPSiBa2	33.4	efxeg	0.98	-
11	CUPSiOh3410	35.4	lmxll	3.64	*
12	CUPOhPa4477	39.6	abxcd	2.2	-
13	CUPSiBa3902	40.3	lmxll	3.28	*
14	CUPOhPa3944	42.2	abxcd	1.26	-
15	CUPVA105	47.2	lmxll	0.83	-
16	CUPVB306	53.3	lmxll	1.58	-
17	CUPVD581	56.5	lmxll	0.85	-
18	CUPOhPa293	71.5	lmxll	0.52	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG4 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPVD309	0.0	lmxll	23.14	*******
2	CUPBaPa989	13.9	efxeg	33.7	*******
3	CUPBaPa570	26.7	abxcd	6.93	*
4	CUPOhPa4043	28.9	lmxll	1.37	-
5	CUPSiBa3508	50.9	abxcd	2.97	-
6	CUPBaPa667	55.6	abxcd	7.97	**
7	CUPOhBa2127	62.1	abxcd	1.68	-
8	CUPSiPa185	65.8	efxeg	7.29	*
9	CUPOhPa4213	69.5	abxcd	5.45	-
10	CUPSiBa4038	72.8	efxeg	2.2	-
11	CUPBaPa2258	77.4	efxeg	1.56	-
12	CUPSiOh2424	88.9	abxcd	2.3	-
13	CUPVB492	90.3	abxcd	2.65	-
14	PVGH271591b	91.6	lmxll	0.81	-
15	PVGH271591c	93.0	lmxll	1	-
16	CUPBaPa1593	93.9	abxcd	1.37	-
17	CUPSiOh4182	98.0	efxeg	0.88	-
18	PVGH271428	101.0	lmxll	0.13	-
19	CUPVSiirt1164	109.0	abxcd	0.56	-
20	CUPSiPa1297	117.4	abxcd	2.18	-
21	CUPSiPa2316	124.8	abxcd	1.43	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG5 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPVSiirt76	0.0	abxcd	25.15	*******
2	CUPOhBa2763	12.2	abxcd	4.32	-
3	CUPSiOh2286	19.8	abxcd	3.94	-
4	CUPVSiirt1672	29.4	abxcd	0.33	-
5	CUPSiBa1336	35.0	abxcd	1.88	-
6	Pislen526	46.5	lmxll	0.03	-
7	CUPOhBa4461	49.9	lmxll	0.52	-
8	CUPVSiirt956	52.6	abxcd	2.65	-
9	CUPSiBa3305	55.9	abxcd	9.02	**
10	PVGH271079a	61.9	lmxll	2.1	-
11	CUPSiPa483	62.9	hkxhk	3.03	-
12	CUPSiOh2679	63.1	lmxll	3.23	*
13	CUPVSiirt543	63.8	efxeg	3.86	-
14	CUPVSiirt37	67.0	abxcd	3.53	-
15	CUPSiPa1556	70.0	abxcd	8.88	**
16	CUPBaPa2294	71.1	abxcd	5.94	-
17	CUPVB630	71.7	lmxll	2.31	-
18	CUPVSiirt1350	71.7	lmxll	2.89	*
19	CUPSiOh746	71.7	lmxll	2.61	-
20	CUPOhPa3954	74.1	efxeg	13.23	****
21	CUPBaPa996	76.1	abxcd	10.5	**
22	CUPBaPa2683	77.6	abxcd	9.56	**
23	CUPOhBa4756	80.4	abxcd	4.5	-
24	CUPOhPa4756	85.4	abxcd	14.52	****
25	CUPOhBa4151	92.9	abxcd	7.14	*
26	CUPSiPa2184	96.0	abxcd	8.19	**
27	CUPOhBa137	99.9	abxcd	13.29	****
28	CUPVB461	103.2	lmxll	8.21	****

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG6 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPVD205	0.0	efxeg	27.26	*******
2	CUPSiOh1824	37.1	abxcd	5.21	-
3	CUPVB577	49.9	abxcd	2.63	-
4	CUPSiBa2540	56.8	abxcd	4.32	-
5	CUPOhPa2472	60.2	efxeg	4.31	-
6	CUPSiBa3286	63.0	abxcd	2.61	-
7	CUPOhBa4503	64.9	abxcd	1.94	-
8	CUPSiPa3378	68.7	lmxll	3.53	*
9	CUPOhPa4538	70.8	lmxll	1.8	-
10	CUPOhBa1966	75.1	abxcd	7.4	*
11	CUPVA318	82.9	lmxll	4.3	**
12	PVGH271186	87.0	lmxll	6.22	**
13	CUPSiOh4085	88.4	abxcd	11.8	***
14	CUPVSiirt876	91.4	abxcd	11.05	**
15	PKAG020	102.4	lmxll	2.06	-
16	CUPVSiirt875	123.2	abxcd	82.81	*******

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG7 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPVSiirt1099	0.0	lmxll	1.16	-
2	CUPSiBa3990	2.6	abxcd	5.05	-
3	CUPSiPa2152	14.5	hkxhk	0.05	-
4	CUPOhPa819	19.9	hkxhk	1.96	-
5	CUPVD521	36.5	abxcd	11.66	***
6	CUPVSiirt1117	47.4	abxcd	6.27	*
7	CUPSiOh4114	55.5	lmxll	1.57	-
8	CUPSiBa3950	62.6	abxcd	3.29	-
9	CUPVSiirt874	64.1	abxcd	1.56	-
10	CUPVSiirt110	68.5	abxcd	2.07	-
11	CUPVB685	70.1	lmxll	1.83	-
12	CUPOhPa1967	71.8	abxcd	7.14	*
13	CUPSiOh1576	73.2	lmxll	2.61	-
14	CUPSiOh2751	73.4	abxcd	4.32	-
15	CUPSiBa1245	74.1	abxcd	4.71	-
16	CUPVSiirt719	77.6	abxcd	0.54	-
17	CUPOhPa4736	80.9	efxeg	3.6	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG8 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X2	Signifcance
1	CUPVSiirt944	0.0	lmxll	3.64	*
2	CUPSiBa3937	5.0	abxcd	2.75	-
3	CUPVB446	14.1	efxeg	7.94	**
4	CUPSiOh2517	19.9	abxcd	2.26	-
5	CUPSiOh4567	29.2	efxeg	2.01	-
6	CUPVA220	32.9	abxcd	6.7	*
7	CUPSiPa2132	34.9	abxcd	6.26	*
8	CUPVB420	37.0	abxcd	7.87	**
9	CUPVSiirt11102	39.8	efxeg	4.06	-
10	CUPSiBa2465	51.5	abxcd	9.03	**
11	CUPSiBa2559	56.0	efxeg	4.26	-
12	CUPSiBa4072	59.8	abxcd	3.86	-
13	CUPOhPa650	61.0	abxcd	5.27	-
14	CUPSiOh527	64.3	lmxll	2.93	*
15	CUPVSiirt1122	65.7	lmxll	0.42	-
16	CUPSiOh1422	77.9	abxcd	13.74	****

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG9 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcane
1	CUPSiOh3552	0.0	abxcd	2.72	-
2	CUPVB571	4.3	abxcd	2.2	-
3	CUPSiOh2560	12.4	abxcd	7.18	*
4	CUPVSiirt1402	20.8	abxcd	6.04	-
5	CUPVSiirt186	29.0	abxcd	3.77	-
6	CUPOhBa1852	32.7	abxcd	3.03	-
7	CUPSiBa3091	33.6	abxcd	4.63	-
8	CUPVSiirt1537	34.5	lmxll	2.31	-
9	CUPVSiirt15	40.9	abxcd	2.34	-
10	CUPSiBa3409	44.7	efxeg	5.17	-
11	CUPSiOh962	45.9	efxeg	5.08	-
12	CUPSiBa4227	50.3	abxcd	6.95	*
13	CUPVB629	52.0	abxcd	6.53	*
14	PVGH2705606	53.4	lmxll	0.52	-
15	CUPSiPa1835	55.4	abxcd	3.71	-
16	PVGH271546a	57.3	lmxll	0.2	-
17	CUPSiOh4685	58.1	abxcd	5.42	-
18	CUPVSiirt841	61.3	abxcd	0.3	-
19	PVGH270916	63.5	efxeg	1.3	-
20	CUPSiPa47	64.8	abxcd	0.22	-
21	CUPOhBa1099	69.4	abxcd	1.08	-
22	CUPSiBa1077	70.5	abxcd	0.95	-
23	CUPSiOh3795	71.9	abxcd	0.54	-
24	CUPVSiirt1438	73.2	abxcd	0.88	-
25	CUPVSiirt18	73.7	lmxll	0.13	-
26	CUPVB108	76.8	abxcd	2.93	-
27	CUPVSiirt974	81.8	abxcd	3.35	-
28	CUPSiPa3754	92.8	abxcd	3.87	-
29	PVGH271770a	100.4	efxeg	8.02	**
30	CUPSiPa732	104.5	efxeg	7.32	*
31	CUPBaPa2518	116.0	abxcd	16.09	****
32	CUPVB465	129.4	abxcd	7.38	*

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG10 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPSiPa2365	0.0	abxcd	1.37	-
2	CUPBaPa1707	5.6	abxcd	5	-
3	CUPVB101	15.6	lmxll	1.29	-
4	CUPSiOh2142	17.4	abxcd	4.2	-
5	CUPVB657	18.0	lmxll	1.4	-
6	CUPSiBa2144	20.3	abxcd	4.93	-
7	CUPVSiirt1017	20.9	lmxll	39.12	*******
8	CUPVD538	21.7	lmxll	3.17	*
9	CUPVSiirt807	22.4	efxeg	5.61	-
10	CUPSiPa3238	24.7	efxeg	3.16	-
11	CUPSiPa3170	29.4	efxeg	6.76	*
12	CUPVB545	38.7	lmxll	3.23	*
13	CUPVSiirt1250	44.1	lmxll	0.4	-
14	CUPOhPa4338	49.2	abxcd	2.01	-
15	CUPVSiirt1057	51.3	abxcd	1.68	-
16	CUPSiBa4207	52.5	efxeg	2.71	-
17	CUPVSiirt239	55.9	abxcd	1.87	-
18	CUPSiOh2976	58.5	lmxll	0.66	-
19	CUPVB208	64.1	abxcd	2.52	-
20	CUPSiBa1130	67.7	lmxll	0.3	-
21	CUPSiPa3387	72.1	abxcd	1.34	-
22	PW008	87.8	lmxll	0.39	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG11 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPSiOh1097	0.0	abxcd	5.37	-
2	CUPSiBa3853	6.1	abxcd	10.47	**
3	CUPSiBa2591	9.1	abxcd	5.81	-
4	CUPSiOh1163	12.0	abxcd	8.93	**
5	CUPVSiirt951	12.7	lmxll	3.45	*
6	CUPVB410	14.4	lmxll	0.03	-
7	CUPVB696	15.8	lmxll	3.59	*
8	CUPVSiirt1153	23.8	abxcd	4.89	-
9	CUPSiBa1152	28.6	abxcd	6.5	*
10	CUPSiOh4084	49.5	abxcd	7.49	*
11	CUPSiPa4715	52.3	lmxll	3.17	*
12	CUPSiOh1195	58.7	lmxll	1.03	-
13	CUPBaPa2540	71.4	lmxll	0.07	-
14	CUPSiOh245	76.1	lmxll	0.67	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG12 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPOhBa1030	0.0	abxcd	1.67	-
2	PVGH270571	4.7	efxeg	3.03	-
3	CUPVB415	9.3	lmxll	0.2	-
4	CUPVD414	20.1	lmxll	0.2	-
5	CUPSiBa812	24.7	lmxll	0.13	-
6	CUPSiPa813	27.4	abxcd	4.5	-
7	CUPSiOh3438	39.9	lmxll	0.41	-
8	CUPVSiirt149	42.2	lmxll	0.2	-
9	CUPSiBa3942	45.2	lmxll	0.07	-
10	CUPSiPa3809	47.9	lmxll	0.07	-
11	CUPSiOh3917	51.9	abxcd	3.31	-
12	CUPSiBa1484	56.6	abxcd	3.48	-
13	CUPVSiirt1488	58.5	abxcd	5.54	-
14	CUPOhBa4344	66.0	abxcd	3.33	-
15	CUPSiOh1704	87.3	lmxll	4.45	**
16	CUPOhBa2293	91.2	lmxll	0.13	-
17	CUPSiOh4652	103.8	lmxll	0.32	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG13 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	PW047	0.0	lmxll	0.08	-
2	CUPVSiirt1481	9.6	abxcd	7.81	*
3	CUPVSiirt1482	16.8	lmxll	0.66	-
4	CUPSiOh3227	23.1	abxcd	0.89	-
5	CUPVSiirt1214	26.4	abxcd	0.19	-
6	CUPSiOh2635	28.0	abxcd	0.16	-
7	CUPSiPa2619	29.8	abxcd	0.92	-
8	CUPVSiirt625	31.4	abxcd	1.3	-
9	CUPBaPa1485	34.1	abxcd	1.3	-
10	CUPSiOh2311	37.4	abxcd	0.56	-
11	CUPVSiirt1075	38.0	lmxll	0.04	-
12	CUPVSiirt1021	43.5	abxcd	1.1	-
13	CUPSiOh3104	46.0	lmxll	0.21	-
14	CUPVD207	51.8	lmxll	2.06	-
15	CUPSiOh4281	58.8	abxcd	1.43	-
16	CUPSiBa4037	59.4	abxcd	2.14	-
17	PVGH271280	60.1	efxeg	2.71	-
18	CUPSiBa3914	63.3	abxcd	3.16	-
19	CUPSiBa825	65.3	lmxll	1.83	-
20	CUPVSiirt1326	67.9	abxcd	3.1	-
21	PKATG018	72.8	lmxll	3.33	*
22	CUPSiBa3976	74.5	efxeg	2.91	-
23	CUPVSiirt363	84.5	efxeg	4.49	-
24	CUPVSiirt1170	90.5	abxcd	1.88	-
25	CUPVSiirt565	96.8	abxcd	3.21	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG14 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPOhBa281	0.0	abxcd	2.39	-
2	CUPBaPa1690	4.8	efxeg	1.94	-
3	CUPVB601	6.5	abxcd	2.26	-
4	CUPBaPa339	24.0	abxcd	0.97	-
5	CUPVSiirt1384	31.9	abxcd	4.71	-
6	CUPSiPa1580	35.8	efxeg	6.9	*
7	CUPOhBa1287	39.5	efxeg	4.13	-
8	CUPBaPa539	46.0	abxcd	1.5	-
9	CUPOhPa2359	53.0	abxcd	2.67	-
10	CUPSiOh4074	63.5	abxcd	2.58	-
11	CUPVSiirt129	65.6	abxcd	1.34	-
12	CUPVSiirt1321	67.8	abxcd	1.18	-
13	CUPVSiirt1655	69.4	lmxll	0.13	-
14	CUPSiBa2648	73.2	efxeg	3.2	-
15	CUPOhBa546	90.0	abxcd	0.6	-
16	CUPOhBa791	117.1	abxcd	1.17	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG15 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Signifcance
1	CUPOhPa4270	0.0	abxcd	6.14	-
2	CUPSiOh2241	5.6	abxcd	8.5	**
3	CUPVb470	11.6	lmxll	7.14	***
4	CUPSiBa426	14.6	lmxll	8.71	****
5	CUPVB583	15.9	lmxll	10.95	*****
6	CUPVSiirt720	16.7	lmxll	7.19	***
7	CUPVB009	19.6	abxcd	8.55	**
8	CUPOhBa2681	23.2	abxcd	9.88	**
9	CUPSiOh1566	24.0	lmxll	4.65	**
10	CUPVB679	25.6	abxcd	12.98	****
11	CUPSiOh1938	26.1	lmxll	8.39	****
12	CUPSiPa3296	26.3	lmxll	6.95	***
13	CUPOhPa2632	26.5	abxcd	9.56	**
14	CUPVSiirt1155	26.7	abxcd	8.89	**
15	CUPVSiirt446	27.6	efxeg	10.39	**
16	CUPOhPa2243	28.6	abxcd	12.06	***
17	CUPSiPa1782	29.4	lmxll	9.8	****
18	CUPVSiirt1246	30.0	abxcd	16.82	*****
19	CUPSiOh3348	30.7	lmxll	8.13	****
20	CUPVSiirt54	32.7	efxeg	21.4	*******
21	CUPSiOh2456	53.0	lmxll	7.14	***
22	CUPVB615	65.8	lmxll	2.7	-
23	CUPOhPa659	80.6	lmxll	0.53	-

SUPPLEMENTARY FILE-2

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG1 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPOhBa473	0	abxcd	4.18	-
2	CUPSiOh4563	3.1	abxcd	0.88	-
3	CUPSiPa1067	5.2	abxcd	4.73	-
4	CUPOhBa2112	6.8	nnxnp	0.2	-
5	CUPSiBa2293	7.9	efxeg	1.1	-
6	CUPOhPa4233	8.5	abxcd	0.8	-
7	CUPVD659	9	nnxnp	0.39	-
8	CUPSiOh303	10.5	nnxnp	0.21	-
9	CUPVB534b	10.8	nnxnp	0.01	-
10	CUPOhPa4118	11.1	nnxnp	0.03	-
11	CUPSiBa3493	11.5	abxcd	0.18	-
12	CUPSiOh2688	11.9	abxcd	0.19	-
13	CUPSiOh52	13	efxeg	0.41	-
14	PVGH271159	13.9	nnxnp	0.07	-
15	CUPSiPa2106	15.8	abxcd	0.35	-
16	CUPSiBa3210	17.4	abxcd	1.85	-
17	CUPSiOh2753	23.4	nnxnp	4.3	**

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG2 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPSiOh952	0.0	abxcd	30.63	*******
2	CUPOhPa923	4.9	nnxnp	0.4	-
3	CUPSiOh3646	5.1	abxcd	4.13	-
4	CUPVD661	19.6	hkxhk	2.58	-
5	PVGH271046	20.8	nnxnp	2.89	*
6	CUPVSiirt259	39.2	abxcd	2	-
7	CUPBaPa108	44.0	efxeg	4.62	-
8	CUPVSiirt1417	46.8	hkxhk	8.34	**
9	CUPOhPa2145	52.3	abxcd	5.72	-
10	CUPOhPa590	61.7	nnxnp	0.01	-
11	CUPSiPa4054	65.5	abxcd	1.74	-
12	CUPVB595	71.7	abxcd	7.71	*
13	CUPSiOh4004	74.9	abxcd	6.63	*
14	CUPVD201	82.7	abxcd	9.65	**
15	CUPSiBa2682	85.9	efxeg	5.72	-
16	CUPSiPa4275	88.3	nnxnp	0.13	-
17	CUPBaPa1646	89.9	nnxnp	0.04	-
18	CUPSiOh3717	91.1	abxcd	11.46	***
19	CUPVB219	102.6	abxcd	27	*******
20	CUPSiPa4413	116.5	nnxnp	0	-
21	PVGH271106	140.7	nnxnp	0.03	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG3 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPSiBa575	0.0	nnxnp	9.8	****
2	CUPSiBa3248	11.2	nnxnp	2.89	*
3	CUPSiBa1284	24.8	abxcd	2.39	-
4	CUPOhPa3361	30.6	abxcd	3.4	-
5	CUPSiOh390	33.7	abxcd	2.01	-
6	CUPSiBa374	35.7	abxcd	2.43	-
7	CUPOhPa152	37.4	nnxnp	0.22	-
8	CUPSiBa2	38.3	efxeg	0.98	-
9	CUPVSiirt136	39.7	nnxnp	0.01	-
10	CUPSiBa4598	41.2	nnxnp	0.54	-
11	CUPOhPa4477	45.2	abxcd	2.2	-
12	CUPOhPa3944	48.2	abxcd	1.26	-
13	CUPVB486	50.4	nnxnp	1.14	-
14	CUPSiOh1443	54.7	nnxnp	2.09	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG4 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPBaPa989	0.0	efxeg	33.7	*******
2	CUPBaPa570	20.2	abxcd	6.93	*
3	CUPSiPa124	34.7	nnxnp	0.13	-
4	CUPSiBa3508	44.0	abxcd	2.97	-
5	CUPBaPa667	48.4	abxcd	7.97	**
6	CUPOhBa2127	54.6	abxcd	1.68	-
7	CUPSiPa185	58.4	efxeg	7.29	*
8	CUPOhPa4213	62.0	abxcd	5.45	-
9	CUPSiBa4038	65.0	efxeg	2.2	-
10	CUPBaPa394	69.0	nnxnp	0.08	-
11	CUPBaPa2258	69.9	efxeg	1.56	-
12	CUPVB492	80.8	abxcd	2.65	-
13	CUPSiOh2424	83.0	abxcd	2.3	-
14	PVGH271703b	84.5	nnxnp	0.07	-
15	CUPBaPa1593	86.1	abxcd	1.37	-
16	CUPSiOh4182	90.2	efxeg	0.88	-
17	CUPVSiirt1164	100.6	abxcd	0.56	-
18	CUPSiPa1297	109.2	abxcd	2.18	-
19	CUPSiPa2316	116.7	abxcd	1.43	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG5 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPVSiirt76	0.0	abxcd	25.15	*******
2	CUPOhBa2763	12.3	abxcd	4.32	-
3	CUPSiOh2286	19.9	abxcd	3.94	-
4	CUPVSiirt1672	29.9	abxcd	0.33	-
5	CUPSiBa1336	35.3	abxcd	1.88	-
6	CUPVSiirt956	51.9	abxcd	2.65	-
7	CUPSiBa3305	55.9	abxcd	9.02	**
8	CUPSiPa483	62.6	hkxhk	3.03	-
9	CUPVSiirt543	63.7	efxeg	3.86	-
10	CUPVSiirt37	66.9	abxcd	3.53	-
11	CUPBaPa1610	68.9	nnxnp	2.31	-
12	CUPSiPa1556	70.1	abxcd	8.88	**
13	CUPBaPa2294	71.2	abxcd	5.94	-
14	CUPOhPa3954	73.5	efxeg	13.23	****
15	CUPBaPa996	75.4	abxcd	10.5	**
16	CUPVD450	76.1	nnxnp	0.97	-
17	CUPBaPa2683	77.3	abxcd	9.56	**
18	CUPOhBa4756	79.8	abxcd	4.5	-
19	CUPOhPa4756	86.2	abxcd	14.52	****
20	CUPOhBa4151	92.5	abxcd	7.14	*
21	CUPSiPa2184	95.6	abxcd	8.19	**
22	CUPOhBa137	99.7	abxcd	13.29	****

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG6 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPVD205	0.0	efxeg	27.26	*******
2	CUPVD423	18.3	nnxnp	10.29	****
3	CUPSiOh1824	37.1	abxcd	5.21	-
4	CUPSiBa1541	44.8	nnxnp	1.35	-
5	CUPVB577	50.5	abxcd	2.63	-
6	CUPBaPa1027	55.1	nnxnp	2.17	-
7	CUPSiBa2540	57.4	abxcd	4.32	-
8	CUPOhPa4532	58.9	nnxnp	2.43	-
9	PVGH271023c	60.8	nnxnp	0.65	-
10	CUPOhPa2472	61.9	efxeg	4.31	-
11	CUPOhBa377	63.7	nnxnp	2.61	-
12	CUPSiBa3286	64.7	abxcd	2.61	-
13	CUPOhBa4503	66.4	abxcd	1.94	-
14	CUPSiPa518	66.9	nnxnp	0.53	-
15	CUPSiPa1592	71.2	nnxnp	7.5	***
16	CUPBaPa992	75.6	nnxnp	1.96	-
17	CUPOhBa1966	76.9	abxcd	7.4	*
18	CUPSiOh4085	89.2	abxcd	11.8	***
19	CUPVSiirt876	92.9	abxcd	11.05	**
20	CUPVSiirt875	123.2	abxcd	82.81	*******

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG7 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Locus name	Position	Segregation	X^2	Significance
1	CUPSiBa3990	0.0	abxcd	5.05	-
2	CUPSiPa2152	11.6	hkxhk	0.05	-
3	CUPOhPa819	16.9	hkxhk	1.96	-
4	CUPVD521	32.4	abxcd	11.66	***
5	CUPSiPa1473	43.7	nnxnp	0.97	-
6	CUPBaPa366	45.6	nnxnp	0.65	-
7	CUPVSiirt1117	47.3	abxcd	6.27	*
8	CUPOhPa1346	50.5	nnxnp	0.4	-
9	CUPBaPa317	54.7	nnxnp	1.14	-
10	CUPOhPa4736	66.3	efxeg	3.6	-
11	CUPVSiirt719	70.0	abxcd	0.54	-
12	CUPOhBa4119	71.8	nnxnp	0.13	-
13	CUPSiOh3994	73.0	nnxnp	0.83	-
14	CUPSiOh2751	73.5	abxcd	4.32	-
15	CUPSiBa1245	74.3	abxcd	4.71	-
16	CUPOhPa1967	75.9	abxcd	7.14	*
17	CUPVSiirt110	78.6	abxcd	2.07	-
18	CUPSiPa2556	79.5	nnxnp	0.3	-
19	CUPVSiirt874	82.9	abxcd	1.56	-
20	CUPSiBa3950	84.1	abxcd	3.29	-
21	PKATG011	88.9	nnxnp	0.3	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG8 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPSiBa3937	0.0	abxcd	2.75	-
2	CUPVB446	9.4	efxeg	7.94	**
3	CUPSiPa4474	13.4	nnxnp	0	-
4	CUPSiOh2517	16.0	abxcd	2.26	-
5	CUPSiOh4567	25.3	efxeg	2.01	-
6	CUPVSiirt11102	28.6	efxeg	4.06	-
7	CUPSiBa4262	29.9	nnxnp	1.14	-
8	CUPSiPa2132	31.2	abxcd	6.26	*
9	CUPVA220	32.6	abxcd	6.7	*
10	CUPVB420	35.5	abxcd	7.87	**
11	CUPSiBa2465	45.6	abxcd	9.03	**
12	CUPOhPa3441	47.9	nnxnp	0.82	-
13	CUPSiBa4072	51.1	abxcd	3.86	-
14	CUPOhPa650	52.5	abxcd	5.27	-
15	CUPSiBa2559	56.1	efxeg	4.26	-
16	CUPSiOh1422	79.0	abxcd	13.74	****

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG9 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPSiOh3552	0.0	abxcd	2.72	-
2	CUPVB571	4.3	abxcd	2.2	-
3	CUPSiOh2560	12.5	abxcd	7.18	*
4	CUPVSiirt1402	21.3	abxcd	6.04	-
5	CUPVSiirt186	29.5	abxcd	3.77	-
6	CUPOhBa1852	33.4	abxcd	3.03	-
7	CUPSiBa3091	34.4	abxcd	4.63	-
8	CUPVSiirt15	42.0	abxcd	2.34	-
9	CUPSiBa3409	46.1	efxeg	5.17	-
10	CUPSiOh962	47.3	efxeg	5.08	-
11	CUPSiBa4227	51.9	abxcd	6.95	*
12	CUPVB629	53.8	abxcd	6.53	*
13	CUPSiPa1835	57.2	abxcd	3.71	-
14	CUPSiOh4685	60.5	abxcd	5.42	-
15	CUPVSiirt841	62.9	abxcd	0.3	-
16	PVGH270916	65.0	efxeg	1.3	-
17	CUPSiPa47	66.4	abxcd	0.22	-
18	CUPOhBa1099	70.7	abxcd	1.08	-
19	CUPSiBa1077	72.0	abxcd	0.95	-
20	CUPSiOh3795	73.4	abxcd	0.54	-
21	CUPVSiirt1438	74.9	abxcd	0.88	-
22	CUPVB108	78.5	abxcd	2.93	-
23	CUPVSiirt974	83.5	abxcd	3.35	-
24	CUPSiPa3754	95.1	abxcd	3.87	-
25	PVGH271770a	102.9	efxeg	8.02	**
26	CUPSiPa732	107.0	efxeg	7.32	*
27	CUPBaPa2518	118.6	abxcd	16.09	****
28	CUPVB465	131.9	abxcd	7.38	*

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG10 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPSiPa1654	0.0	nnxnp	0.79	-
2	CUPSiPa2365	6.6	abxcd	1.37	-
3	CUPBaPa1707	12.4	abxcd	5	-
4	CUPSiOh2142	24.9	abxcd	4.2	-
5	CUPSiBa2144	27.6	abxcd	4.93	-
6	CUPVSiirt807	29.7	efxeg	5.61	-
7	CUPSiPa233	30.9	nnxnp	0.13	-
8	CUPSiPa3238	32.1	efxeg	3.16	-
9	CUPSiPa3170	36.9	efxeg	6.76	*
10	CUPBaPa299	41.4	nnxnp	0.03	-
11	CUPVSiirt82	50.4	nnxnp	0.54	-
12	CUPOhPa4338	59.5	abxcd	2.01	-
13	CUPVSiirt1057	61.8	abxcd	1.68	-
14	CUPSiBa4207	63.1	efxeg	2.71	-
15	CUPVSiirt239	66.7	abxcd	1.87	-
16	CUPVB208	75.1	abxcd	2.52	-
17	CUPSiPa3387	83.2	abxcd	1.34	-
18	CUPSiOh2251	98.4	nnxnp	1.18	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG11 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Locus name	Position	Segregation	X^2	Significance
1	CUPBaPa1220	0.0	nnxnp	0.03	-
2	CUPSiOh1518	5.6	nnxnp	0	-
3	CUPSiOh1097	12.4	abxcd	5.37	-
4	CUPSiBa3853	17.6	abxcd	10.47	**
5	CUPBaPa934	19.8	nnxnp	2.35	-
6	CUPSiBa2591	21.0	abxcd	5.81	-
7	CUPBaPa826	21.9	nnxnp	2.57	-
8	CUPSiOh1163	24.1	abxcd	8.93	**
9	CUPVSiirt1153	34.3	abxcd	4.89	-
10	CUPSiBa1152	38.9	abxcd	6.5	*
11	CUPSiBa2111	43.0	nnxnp	0.13	-
12	CUPOhBa4362	53.0	nnxnp	0.21	-
13	CUPSiOh4084	60.7	abxcd	7.49	*
14	CUPSiBa1254	72.4	nnxnp	0.39	-
15	CUPSiBa1254	73.4	nnxnp	0.39	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG12 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	PVGH270571	0.0	efxeg	3.03	-
2	CUPOhBa1030	5.0	abxcd	1.67	-
3	Ptms-41	8.2	nnxnp	0.4	-
4	CUPSiBa1991	20.1	nnxnp	1.66	-
5	CUPSiPa813	26.1	abxcd	4.5	-
6	CUPSiOh4234	35.6	nnxnp	0.66	-
7	Ptms-3	40.6	nnxnp	0	-
8	CUPSiPa4645	42.3	nnxnp	0.52	-
9	CUPSiPa4647	43.9	nnxnp	0.53	-
10	CUPSiOh4133	50.6	nnxnp	1.35	-
11	CUPSiPa1741	51.9	nnxnp	2.51	-
12	CUPSiOh3917	56.9	abxcd	3.31	-
13	CUPSiOh1403	60.4	nnxnp	2.75	*
14	CUPSiBa1484	62.0	abxcd	3.48	-
15	CUPVSiirt1488	63.9	abxcd	5.54	-
16	CUPSiPa3464	64.7	nnxnp	6.84	***
17	CUPOhBa4344	73.0	abxcd	3.33	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG13 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPVSiirt1481	0.0	abxcd	7.81	*
2	CUPOhPa1575	14.4	nnxnp	0.14	-
3	CUPSiOh3227	18.7	abxcd	0.89	-
4	CUPSiPa474	21.2	nnxnp	0.13	-
5	CUPVSiirt1214	22.3	abxcd	0.19	-
6	CUPSiOh2635	23.7	abxcd	0.16	-
7	CUPSiPa2619	25.8	abxcd	0.92	-
8	CUPVSiirt625	27.5	abxcd	1.3	-
9	CUPBaPa1485	29.9	abxcd	1.3	-
10	CUPSiOh2311	33.6	abxcd	0.56	-
11	CUPVSiirt1021	40.2	abxcd	1.1	-
12	CUPSiPa1653	44.7	nnxnp	0.29	-
13	CUPSiPa3801	53.4	nnxnp	0.67	-
14	PVGH271280	57.1	efxeg	2.71	-
15	CUPSiBa4037	57.7	abxcd	2.14	-
16	CUPSiOh4281	58.3	abxcd	1.43	-
17	CUPSiBa3914	61.8	abxcd	3.16	-
18	CUPVSiirt1326	66.4	abxcd	3.1	-
19	CUPSiBa3976	72.2	efxeg	2.91	-
20	CUPSiBa3312	77.8	nnxnp	0.13	-
21	CUPVSiirt363	83.0	efxeg	4.49	-
22	CUPVSiirt1170	89.1	abxcd	1.88	-
23	CUPVSiirt565	95.4	abxcd	3.21	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG14 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPOhBa281	0.0	abxcd	2.39	-
2	CUPBaPa1690	4.8	efxeg	1.94	-
3	CUPVB601	6.5	abxcd	2.26	-
4	CUPBaPa339	23.9	abxcd	0.97	-
5	CUPVSiirt1384	32.0	abxcd	4.71	-
6	CUPSiPa1580	35.9	efxeg	6.9	*
7	CUPOhBa1287	39.5	efxeg	4.13	-
8	CUPBaPa539	46.0	abxcd	1.5	-
9	CUPOhPa2359	52.9	abxcd	2.67	-
10	PKAG005	59.6	nnxnp	0.65	-
11	CUPSiOh4074	64.5	abxcd	2.58	-
12	CUPVSiirt129	66.3	abxcd	1.34	-
13	CUPBaPa87	67.5	nnxnp	1.56	-
14	CUPVSiirt1321	68.3	abxcd	1.18	-
15	PVGH271139a	68.7	nnxnp	0.98	-
16	CUPSiBa2648	74.3	efxeg	3.2	-
17	CUPOhBa546	88.0	abxcd	0.6	-
18	CUPOhBa791	115.5	abxcd	1.17	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR loci in LG15 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	CUPSiBa901	0.0	nnxnp	0	-
2	CUPOhPa4270	2.4	abxcd	6.14	-
3	CUPSiOh2241	7.1	abxcd	8.5	**
4	CUPOhPa2458	9.8	nnxnp	0.08	-
5	CUPSiBa4070	11.6	nnxnp	0.03	-
6	CUPSiPa2938	15.5	nnxnp	0.52	-
7	CUPOhPa3049	17.6	nnxnp	0.83	-
8	CUPVB009	22.8	abxcd	8.55	**
9	CUPOhBa2681	27.1	abxcd	9.88	**
10	CUPVB679	29.2	abxcd	12.98	****
11	CUPOhPa2632	30.0	abxcd	9.56	**
12	CUPVSiirt1155	30.4	abxcd	8.89	**
13	CUPVSiirt446	31.3	efxeg	10.39	**
14	CUPOhPa2243	32.4	abxcd	12.06	***
15	CUPVSiirt1246	33.9	abxcd	16.82	*****
16	CUPVSiirt54	36.3	efxeg	21.4	*******
17	CUPBaPa931	49.6	nnxnp	0.82	-

SUPPLEMENTARY FILE-3

Primer name, LG, positions (cM), Segregation, Significant values, Zigotic Maternal and Paternal distortion of SSR loci in 'Siirt x Pa-18' F1 population

Primer name	Position	Segregation	Significant	Zigot distortion	Maternal distortion	Paternal distortion
CUPSiOh2753	41.637	[nn:np]	**	1.0000		0.0381
CUPVB219	38.044	[ac:ad:bc:bd]	*******	0.1623	0.0001	0.0739
CUPSiOh3717	49.538	[ac:ad:bc:bd]	***	1.0000	0.0007	1.0000
CUPVD201	57.997	[ac:ad:bc:bd]	**	0.8563	0.0064	0.1582
CUPSiOh4004	65.763	[ac:ad:bc:bd]	*	0.9745	0.0126	0.7216
CUPVB595	68.925	[ac:ad:bc:bd]	*	0.9813	0.0075	0.5930
CUPSiOh2178	86.858	[ll:lm]	**	1.0000	0.0482	
CUPVB463	91.643	[ll:lm]	**	1.0000	0.0311	
CUPVSiirt1417	94.414	[hh:hk:kk]	**	0.3403	0.1018	0.1018
PVGH270917	94.789	[ll:lm]	*	1.0000	0.0725	
CUPSiOh2689	105.645	[ll:lm]	**	1.0000	0.0271	
PVGH271046	121.186	[nn:np]	*	1.0000		0.0892
CUPSiBa575	0	[nn:np]	****	1.0000		0.0017
CUPSiBa3248	11.178	[nn:np]	*	1.0000		0.0892
CUPBaPa739	33.292	[ll:lm]	**	1.0000	0.0500	
CUPSiOh3410	40.806	[ll:lm]	*	1.0000	0.0563	
CUPSiBa3902	45.77	[ll:lm]	*	1.0000	0.0702	
CUPVD309	0	[ll:lm]	*******	1.0000	0.0000	
CUPBaPa989	13.943	[ee:ef:eg:fg]	*******	0.0000	0.5637	0.8474
CUPBaPa570	27.356	[ac:ad:bc:bd]	*	0.5180	0.0285	1.0000
CUPBaPa667	55.641	[ac:ad:bc:bd]	**	0.2497	0.0464	0.8563
CUPSiPa185	65.534	[ee:ef:eg:fg]	*	0.0561	0.6467	0.9270
CUPVSiirt76	0	[ac:ad:bc:bd]	*******	0.5796	0.0000	0.7851
CUPSiBa3305	55.795	[ac:ad:bc:bd]	**	0.2704	0.0563	0.3173
CUPSiOh2679	62.837	[ll:lm]	*	1.0000	0.0725	
CUPSiPa1556	69.644	[ac:ad:bc:bd]	**	0.1948	0.1727	0.1727
CUPVSiirt1350	71.453	[ll:lm]	*	1.0000	0.0892	

FILE-3. Continued

CUPOhPa3954	73.811	[ee:ef:eg:fg]	****	0.4086	0.0071	0.1508
CUPBaPa996	75.732	[ac:ad:bc:bd]	**	0.4223	0.0242	0.1762
CUPBaPa2683	77.143	[ac:ad:bc:bd]	**	0.3119	0.0603	0.1797
CUPOhPa4756	85.416	[ac:ad:bc:bd]	****	0.9240	0.0522	0.0023
CUPOhBa4151	92.434	[ac:ad:bc:bd]	*	0.9919	0.0325	0.1088
CUPSiPa2184	95.425	[ac:ad:bc:bd]	**	0.5791	0.0196	0.2087
CUPOhBa137	99.276	[ac:ad:bc:bd]	****	0.6422	0.0052	0.0242
CUPVB461	102.771	[ll:lm]	****	1.0000	0.0042	
CUPVD205	0	[ee:ef:eg:fg]	*******	0.0000	0.0008	0.4133
CUPVD423	18.317	[nn:np]	****	1.0000		0.0013
CUPSiPa3378	69.506	[ll:lm]	*	1.0000	0.0603	
CUPSiPa1592	69.908	[nn:np]	***	1.0000		0.0062
CUPOhBa1966	76.519	[ac:ad:bc:bd]	*	0.0810	0.8586	0.2850
CUPVA318	83.623	[ll:lm]	**	1.0000	0.0381	
PVGH271186	87.692	[ll:lm]	**	1.0000	0.0126	
CUPSiOh4085	89.027	[ac:ad:bc:bd]	***	0.9999	0.0017	0.1797
CUPVSiirt876	91.971	[ac:ad:bc:bd]	**	0.8590	0.0298	0.0298
CUPVSiirt875	122.565	[ac:ad:bc:bd]	*******	0.0000	0.0000	0.0205
CUPVD521	34.351	[ac:ad:bc:bd]	***	0.9457	0.4094	0.0013
CUPVSiirt1117	47.772	[ac:ad:bc:bd]	*	0.1613	0.6521	0.3213
CUPOhPa1967	75.193	[ac:ad:bc:bd]	*	0.9239	0.0500	0.0748
CUPVSiirt944	0	[ll:lm]	*	1.0000	0.0563	
CUPVB446	13.792	[ee:ef:eg:fg]	**	0.0840	0.2087	0.8575
CUPSiPa2132	35.559	[ac:ad:bc:bd]	*	0.9840	0.0196	0.4725
CUPVA220	36.957	[ac:ad:bc:bd]	*	0.9959	0.0197	0.3050
CUPVB420	39.822	[ac:ad:bc:bd]	**	0.8791	0.0311	0.1508
CUPSiBa2465	49.954	[ac:ad:bc:bd]	**	0.5656	0.0633	0.0947
CUPSiOh527	62.884	[ll:lm]	*	1.0000	0.0867	
CUPSiOh1422	76.427	[ac:ad:bc:bd]	****	0.9990	0.2050	0.0006
CUPSiOh2560	12.402	[ac:ad:bc:bd]	*	0.9631	0.0867	0.0583
CUPSiBa4227	50.276	[ac:ad:bc:bd]	*	0.7747	0.2123	0.0500
CUPVB629	51.96	[ac:ad:bc:bd]	*	0.5953	0.1762	0.1253

FILE-3. Continued

PVGH271770a	100.394	[ee:ef:eg:fg]	**	0.4540	0.0365	0.4133
CUPSiPa732	104.471	[ee:ef:eg:fg]	*	0.9036	0.0095	0.6547
CUPBaPa2518	116.04	[ac:ad:bc:bd]	****	0.9999	0.0089	0.0029
CUPVB465	129.371	[ac:ad:bc:bd]	*	0.6461	0.0158	0.5775
CUPVSiirt1017	27.729	[ll:lm]	*******	1.0000	0.0000	
CUPVD538	28.562	[ll:lm]	*	1.0000	0.0748	
CUPSiPa3170	36.116	[ee:ef:eg:fg]	*	0.6652	0.2123	0.0748
CUPVB545	45.949	[ll:lm]	*	1.0000	0.0725	
CUPSiBa3853	17.694	[ac:ad:bc:bd]	**	0.9926	0.0285	0.0176
CUPSiOh1163	23.821	[ac:ad:bc:bd]	**	0.9707	0.0149	0.1253
CUPVSiirt951	24.341	[ll:lm]	*	1.0000	0.0634	
CUPVB696	27.487	[ll:lm]	*	1.0000	0.0583	
CUPSiBa1152	39.156	[ac:ad:bc:bd]	*	0.9488	0.0502	0.1619
CUPSiOh4084	60.619	[ac:ad:bc:bd]	*	0.9035	0.0099	0.4615
CUPSiPa4715	63.408	[ll:lm]	*	1.0000	0.0748	
CUPSiOh1403	57.933	[nn:np]	*	1.0000		0.0975
CUPSiPa3464	62.204	[nn:np]	***	1.0000		0.0089
CUPSiOh1704	89.197	[ll:lm]	**	1.0000	0.0350	
CUPVSiirt1481	9.582	[ac:ad:bc:bd]	*	0.9875	0.4725	0.0071
PKATG018	75.609	[ll:lm]	*	1.0000	0.0679	
CUPSiPa1580	35.824	[ee:ef:eg:fg]	*	0.9802	0.5900	0.0119
CUPSiOh2241	7.251	[ac:ad:bc:bd]	**	0.6605	0.0079	0.9270
CUPVb470	14.659	[ll:lm]	***	1.0000	0.0075	
CUPSiBa426	17.751	[ll:lm]	****	1.0000	0.0032	
CUPVB583	19.028	[ll:lm]	*****	1.0000	0.0009	
CUPVSiirt720	19.853	[ll:lm]	***	1.0000	0.0073	
CUPVB009	23.119	[ac:ad:bc:bd]	**	0.9929	0.0048	0.5245
CUPOhBa2681	26.61	[ac:ad:bc:bd]	**	0.8348	0.0141	0.0563
CUPSiOh1566	27.254	[ll:lm]	**	1.0000	0.0311	
CUPVB679	28.973	[ac:ad:bc:bd]	****	0.7207	0.0044	0.0325
CUPSiOh1938	29.359	[ll:lm]	****	1.0000	0.0038	
CUPSiPa3296	29.553	[ll:lm]	***	1.0000	0.0084	

FILE-3. Continued

CUPOhPa2632	29.718	[ac:ad:bc:bd]	**	0.9413	0.0056	0.1797
CUPVSiirt1155	29.846	[ac:ad:bc:bd]	**	0.9197	0.0114	0.0917
CUPVSiirt446	30.949	[ee:ef:eg:fg]	**	0.7778	0.0196	0.0311
CUPOhPa2243	31.852	[ac:ad:bc:bd]	***	0.9719	0.0141	0.0141
CUPSiPa1782	32.688	[ll:lm]	****	1.0000	0.0017	
CUPVSiirt1246	33.266	[ac:ad:bc:bd]	*****	0.7856	0.0038	0.0038
CUPSiOh3348	33.932	[ll:lm]	****	1.0000	0.0044	
CUPVSiirt54	35.889	[ee:ef:eg:fg]	*******	0.4758	0.0079	0.0002
CUPSiOh2456	56.289	[ll:lm]	***	1.0000	0.0075	

SUPPLEMENTARY FILE-4

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR. SCAR. SNP loci in LG1 Siirt map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	PVF1	0.0	lmxll	24.89	*******
2	CUPVC313	7.4	lmxll	0.07	-
3	CUPVB426	12.4	lmxll	0.03	-
4	SNP-167992	20.9	hkxhk	0.26	-
5	CUPOhBa473	22.8	abxcd	4.18	-
6	SNP-130935	25.4	hkxhk	0.26	-
7	SNP-165792	26.0	efxeg	0.54	-
8	SNP-135862	26.3	lmxll	0.51	-
9	SNP-136404	26.3	lmxll	0.51	-
10	SNP-136228	26.3	lmxll	0.51	-
11	SNP-175177	26.3	lmxll	0.51	-
12	SNP-112277b	26.3	lmxll	0.51	-
13	SNP-130684a	26.3	lmxll	0.51	-
14	SNP-133396	26.3	lmxll	0.51	-
15	SNP-130684b	26.3	lmxll	0.51	-
16	SNP-112277	26.3	lmxll	0.51	-
17	SNP-127343	26.3	lmxll	0.51	-
18	SNP-179354	26.3	lmxll	0.51	-
19	SNP-174431	26.3	lmxll	0.51	-
20	CUPVSiirt1716	26.4	lmxll	0.4	-
21	SNP-133197	26.6	hkxhk	0.52	-
22	CUPSiOh4563	27.7	abxcd	0.88	-
23	CUPSiOh282	30.0	lmxll	0.79	-
24	CUPSiPa1067	30.4	abxcd	4.73	-
25	CUPSiBa2293	32.7	efxeg	1.1	-
26	CUPVSiirt117	32.8	lmxll	0.1	-
27	CUPOhPa4233	33.2	abxcd	0.8	-
28	CUPSiOh2688	36.9	abxcd	0.19	-
29	CUPSiBa3493	37.1	abxcd	0.18	-
30	PVGH271302	37.7	lmxll	0.13	-
31	CUPSiOh52	38.2	efxeg	0.41	-
32	CUPVSiirt472a	38.7	lmxll	0	-
33	CUPVSiirt698	38.9	lmxll	0.01	-
34	CUPSiOh2361	39.4	lmxll	0.03	-
35	CUPSiPa2106	40.3	abxcd	0.35	-
36	CUPSiPa2994	40.8	lmxll	0.4	-
37	CUPSiBa3210	42.6	abxcd	1.85	-
38	CUPVSiirt71	43.4	lmxll	0.04	-
39	CUPSiOh1943	44.2	lmxll	0.03	-

Locus name. positions (cM), Segregation, chi-square values (X^2) and significant levels of SSR. SNP loci in LG1 Pa-18 map of 'Siirt x Pa-18' F1 population.

No	Primer name	Position	Segregation	X^2	Significance
1	SNP-167992	0.0	[hh+hk+h-:kk]	0.26	-
2	CUPOhBa473	2.5	abxcd	4.18	-
3	SNP-130935	3.9	[hh+hk+h-:kk]	0.26	-
4	SNP-165792	4.2	efxeg	0.54	-
5	SNP-133197	5.0	hkxhk	0.52	-
6	CUPSiOh4563	6.5	abxcd	0.88	-
7	CUPSiPa1067	8.5	abxcd	4.73	-
8	CUPOhBa2112	9.8	nnxnp	0.2	-
9	CUPSiBa2293	10.9	efxeg	1.1	-
10	CUPOhPa4233	11.4	abxcd	0.8	-
11	CUPVD659	12.0	nnxnp	0.39	-
12	CUPOhPa4118	12.9	nnxnp	0.03	-
13	CUPVB534b	13.6	nnxnp	0.01	-
14	CUPSiOh303	14.1	nnxnp	0.21	-
15	CUPSiBa3493	14.4	abxcd	0.18	-
16	CUPSiOh2688	14.8	abxcd	0.19	-
17	CUPSiOh52	15.9	efxeg	0.41	-
18	PVGH271159	16.6	nnxnp	0.07	-
19	CUPSiPa2106	18.6	abxcd	0.35	-
20	CUPSiBa3210	20.2	abxcd	1.85	-
21	CUPSiOh2753	26.0	nnxnp	4.3	**