CUKUROVA UNIVERSITY

INSTITUTE OF NATURAL AND APPLIED SCIENCES

Ph.D THESIS

Comparative Metabolome and Transcriptome Analysis of Anthocyanin Biosynthesis in Fig *(Ficus carica* L.)

Ghassan ZAHID

Biotechnology Department

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Advisor:. Prof. Dr. Yıldız AKA KAÇAR

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ABSTRACT

Fig (*Ficus carica* L.), which has vital health-benefiting properties such as anthocyanins, is of paramount importance in the Mediterranean diet. Turkiye is the world's leading producer of figs and recognizes this fruit as a key part of its cuisine. However, despite their diversity and rich medicinal value, local figs are overlooked and poorly examined for the biochemical fingerprints associated with their nutritional quality, and little information is available on their molecular structure beyond fig anthocyanin biosynthesis and coloration. This study was therefore first designed to analyze the peels and pulps of eight Turkish figs for their total phenolic composition, sugars, and antioxidant capacity. Thirty-one phenolic compounds were detected using High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS/MS) method. Among the peels and pulps, the highest mean value of colored and colorless phenolic compounds was found in the '01-1N-58' (dark violet-purple) genotype.

Furthermore, differentially expressed genes involved in the fig anthocyanin mechanism were assessed. For this, RNA-Seq analysis was performed on the peels of the dark-purple '01-1N-58' genotype and greenish-yellowish 'Sarilop' cultivar using Illumina NovaSeq 6000™. Based on these genes, gene ontology (GO) analysis was performed. A total of 21102 differentially expressed genes were observed, and 3542 up-regulated and 3164 down-regulated genes in the libraries of two fig samples were identified. Additionally, qRT-PCR analyses were also conducted to validate the results of the expression of genes in RNA-Seq.

In conclusion; our study showed that the diversity of bioactive compounds in the peels of eight fig cultivars and genotypes is high. It has been demonstrated once again that dark-colored fruits are important for health, and it has been emphasized that the evaluation of fig genetic resources in this context may be important for more effective use.As a result of transcriptome analyzes, important information has been obtained for the determination of anthocyanin-related genes, and it is thought that these data will shed light on future studies for the identification and functional analysis of genes effective in anthocyanin metabolism.

Keywords: Transcriptomic, RNA-Seq, Phenolic compounds, Ilumina, qRT-PCR, HPLC-MS/MS.

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

DOKTORA TEZ ONAYI

İncir (*Ficus carica* L.)'de Antosiyanin Biyosentezinin Karşılaştırmalı Metabolom ve Transkriptom Analizi

Ghassan ZAHID

Danışman:. Prof. Dr. Yıldız AKA KAÇAR

Biyoteknoloji Anabilim Dalı

ÖZ

İncir (*Ficus carica* L.), antosiyaninler gibi sağlığa faydalı bileşiklere sahip olan bir meyve olup, Akdeniz diyetinde büyük önem taşımaktadır. Türkiye dünyanın önde gelen incir üreticisidir ve bu meyvenin Türk mutfağında çok önemli bir yeri vardır. Genetik çeşitliliklerine ve zengin tıbbi değerlerine rağmen, yerel incir genotipleri göz ardı edilmektedir. Besin kaliteleri ile ilişkili biyokimyasal parmakizleri açısından ve incir antosiyanin biyosentezi ile renklenmesinin moleküler mekanizmaları hakkında çok az bilgi bulunmaktadır. Bu nedenle bu çalışma sekiz incir genotipinin kabuklarında ve meyve etindeki toplam fenolik bileşimleri, şekerleri ve antioksidan kapasitelerini analiz etmek ve antosiyaninden sorumlu genlerin transcriptom analizlerini ortaya koymak için tasarlanmıştır. Kütle Spektrometresi (HPLC-MS/MS) yöntemiyle Yüksek Performanslı Sıvı Kromatografisi kullanılarak otuz bir fenolik bileşik tespit edilmiştir. Kabuk ve meyve eti arasında renkli ve renksiz fenolik bileşiklerin ortalama değeri en yüksek '01-1N-58' (koyu mor) genotipinde bulunmuştur. Ayrıca incir antosiyanin mekanizmasında yer alan diferansiyel olarak eksprese edilen genler değerlendirilmiştir. Bunun için Illumina NovaSeq 6000™ dizileme cihazı kullanılarak koyu mor '01-1N-58' genotipi ve 'Sarılop' çeşidinin kabuklarında RNA-Seq analizi yapılmıştır. Elde edilen genlerle gen ontolojisi (GO) analizi yapılmış; toplam 21102 diferansiyel olarak eksprese edilmiş gen tespit edilmiştir. İki incir genotipinin genetik kütüphanelerinde 3542 up-regüle ve 3164 down-regüle gen tanımlanmıştır. Ayrıca, RNA-Seq analizleri sonucunda elde edilen genlerin ekspresyonunu doğrulamak için qRT-PCR analizleri yapılmıştır. Transkriptom analizleri sonucunda antosiyanin ile ilişkili genlerin belirlenmesine yönelik önemli bilgiler elde edilmiş olup, bu verilerin antosiyanin metabolizmasında etkili genlerin tanımlanması ve fonksiyonel analizleri için gelecekteki çalışmalara ışık tutacağı düşünülmektedir.

Anahtar Kelimeler: Transkriptomik, RNA-Seq, Fenolik bileşikler, Ilumina, qRT-PCR, HPLC-MS/MS.

EXTENDED SUMMARY

Ficus carica L., also known as the common fig, is a species of flowering plant in the Moraceae family. It is native to the Mediterranean region and Western Asia and has been cultivated for thousands of years for its fruit, which is a major food source in many parts of the world. The fig is a popular plant for home gardens and is also grown commercially for its fruit and has played an important role in the cultures and cuisines of many countries. Fig trees are well adapted to the Mediterranean climate and have a long history of cultivation in countries such as Türkiye. Fig trees are widely grown in Türkiye for their delicious fruit, which is enjoyed fresh or dried and is also used in a variety of dishes and recipes. In addition to being an important food source, figs and fig trees also have cultural and economic significance in Türkiye. They are often grown in home gardens and orchards, and the fruit is an important source of income for some small farmers. In addition, figs are an important export product for Türkiye, and the country is one of the largest producers and exporters of figs in the world.

Apart from their versatility figs contain a significant amount of phenolic compounds (PCs), sugars, and antioxidants, all of which are important for preventing heart and metabolic diseases, microbial infections, obesity, diabetes, and cancer in humans. These PCs are also renowned for their antioxidant capacity particularly anthocyanins are a type of pigment that is found in a wide range of plant-based foods, including fruits, vegetables, and grains. They are responsible for the red, blue, and purple colors of many foods, and are part of the class of compounds known as flavonoids. Anthocyanins have been the subject of numerous studies due to their potential health benefits. Some potential health benefits of anthocyanins include powerful antioxidants, which means they can help to protect the body's cells from damage caused by free radicals. It may help to reduce the risk of heart disease by improving the function of blood vessels, reducing blood pressure, and lowering cholesterol levels. Anthocyanins may also have a positive effect on cognitive function and memory. Anthocyanins may help to improve insulin sensitivity and blood sugar control in people with diabetes. Moreover, many essential nutrients are found in fruit peels, including fibers, antioxidants, minerals, and vitamins. The formation of anthocyanins and degradation of chlorophyll in the fruit's skin usually begins during the second rapid growth stage, simultaneously with fruit ripening, sugar accumulation and formation of other multiple quality traits. Following anthocyanin, sugar is another significant fruit quality trait. They synchronize the expression of numerous genes and are responsible for the synthesis of polyphenols, organic and amino acids, aroma compounds, as well as pigments.

On the contrary, a High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS) fingerprint is a comprehensive method for assessing food and plant extracts for quality and reliability. As Turkiye is the world's largest producer of figs, and this fruit is recognized as a vital part of the Turkish diet. Despite the great diversity and rich medicinal value, local figs have

been overlooked and remained poorly examined for biochemical fingerprints associated with their nutritional quality. Consequently, the present study was intended to characterize and determine the qualitative and quantitative composition of the differences in the bioactive content in the peels and pulps of eight distinct Turkish commercial fig cultivars and genotypes. A total of four Turkish figs (*F.carica* L.) cultivars 'Sarilop' (greenish-yellow), 'Yesil Bozova' (greenish-light-purple), 'Bursa Siyahi' (dark-purple), 'Izmir Bardakci' (yellowish-green), and four Turkish selected genotypes '01-1N-58' (dark-purple), '31-N-05' (dark-purple), '01-1M-02' (greenish-purple), and '01-1N-51'(dark-purple) were screened for the total phenolic compositions, sugars and total antioxidant capacity. The selected genotypes were collected during their tree ripening stage and analyzed by High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS/MS) method. Fruits were collected at the tree ripening stage in an icebox directly from the orchard of Çukurova University (37°01'46.6''N-35°21'46.6''E), Adana, Turkiye during August and September 2021. At least 20 healthy fruits per cultivar and genotype were selected based on various colors and their economic importance. Soon after, the samples were transported immediately to the molecular laboratory of Çukurova University, Adana, Turkiye within 20 minutes to peel off the selected fig cultivars and genotypes. The fruits were cleaned with 70 % ethanol and then peeled with the help of a scalpel, and the peel was detached from the pulp separately. The skin and pulp samples were immediately wrapped in aluminum foil and dipped in liquid nitrogen, and immediately kept at -20°C and an additional quantity of fig samples were held at -80 °C for later experimental use.

In the first phase (HPLC-MS/MS) study was carried out for the detection of colored-anthocyanins and colorless phenolic compounds PCs. In total, 31 phenolic compounds including 7 anthocyanins (Cyanidin-3, 5-O-diglucoside, (Epi) catechin-(4-8)-cyanidin-3-rutinoside, (Epi) catechin-(4-8)-cyanidin-3-rutinoside, Cyanidin-3-glucoside, Cyanidin-3-O-rutinoside, Pelargonidin 3-0-rutinoside and Cyanidin-3-malonylglucoside) and 24 non-colored phenolics were also detected. Significant differences were found in the anthocyanin content in both peel and pulp, and within cultivars and genotypes. The maximum mean value of anthocyanins in the peel was recorded as 151.25 mg/100 g DW in the '01-1N-58' (dark violet purple) genotype whereas, the minimum mean value was noted as 0.23 mg/100 g DW in 'Sarilop' (green-yellowish) cultivar. Likewise, the maximum amount of anthocyanin mean values in the pulp was documented as 31.57 mg/100 g DW in the '01-1N-58' genotype while the minimum mean value was found as 4.86 mg/100 g DW in 'Sarilop' cultivar, respectively. Among the identified anthocyanins, the percentage of cyanidin derivatives were the most abundant detected both in the peels and the pulps.

The higher amount of the colorless phenolics were computed to be 1063.91 mg GAE/100 g DW in the (01-1N-58') genotype, whereas the minimum mean value was found as 582.17 mg GAE/100 g DW in the peels of the (01-1N-51) genotype, respectively. Furthermore, in our study,

among the identified colorless phenolics, chlorogenic acid (CA) was found to be the dominant compound in the peels of the eight fig cultivars and genotypes, with the total mean values ranging between 91.13-524.74 mg GAE/100 g DW. Likewise, the utmost value was computed as 780.90 mg GAE/100 g DW in (Sarilop) cultivar, whereas the lowest amount of the colorless phenolics were found to be 272.82 mg GAE/100 g DW in the pulp of the fig genotype (31-N-05), respectively. Similar to the peels of the colorless phenolics, chlorogenic acid (CA) was also found to be the dominant compound in the pulp of the eight fig cultivars and genotype as well, with the total mean values ranging between 16.55-363.08 mg GAE/100 g DW.

In the second phase of the study, an HPLC study was carried out for the detection of sugars from the peels and pulps of the eight experimented fig cultivars and genotypes. The maximum mean value of sugars in the skin was recorded as 504.64 g/ kg−1 DW in 'Izmir Bardakci' (yellowish-green) cultivar and a minimum mean value was found as 380.25 g/ kg−1 DW in 'Bursa Siyahi' (dark violet purple) cultivar. Likewise, the highest mean value of total sugars in pulp was documented as 546.94 g/ kg−1 DW in 'Bursa Siyahi' cultivar and the lowest mean value was found as 493.45 g/ kg−1 DW in 'Izmir Bardakci', cultivar. Among the total sugars, glucose was the most abundantly found sugar in the skin, accompanied by fructose and sucrose. Glucose was the predominant sugar with a mean value of 183.24–238.79 g/ kg−1 DW, followed by fructose 173.52–228.81 g/ kg−1 DW and sucrose 23.50–80.62 g/ kg−1 DW, respectively. In the pulp of the fig cultivars and genotypes among the total sugars, fructose was found to be the predominant sugar with a mean value of 225.42-270.33 g/ kg−1 DW, accompanied by glucose 217.53-250.16 g/ kg−1 DW and sucrose 28.07-58.48 g/ kg−1 DW, respectively.

In the third phase of the study, the two assays 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH) and 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were determined to assess the antioxidant activity in both their peels and pulps of eight fig cultivars and genotypes. The fig genotype '01-1N-58' had the highest antioxidant capacity mean values measured by DPPH (28.08 mM Trolox /gdw) while '31-N-05' genotype had the highest ABTS value of (127.34 mM Trolox /gdw), respectively. In contrast, 'SARILOP' cultivar had the lowest antioxidant capacity measured by DPPH (7.84 mM Trolox /gdw) and ABTS (43.81 mM Trolox /gdw), respectively. Whereas, in the pulp, our study depicted that fig genotype '01-1N-58' had the highest antioxidant capacity measured by DPPH (9.41 mM Trolox /gdw) and by ABTS (50.94 mM Trolox /gdw), respectively. However, '31-N-05' genotype had the lowest antioxidant capacity measured by DPPH (4.55 mM Trolox /gdw) and ABTS (28.45mM Trolox /gdw), respectively.

Additionally, for this study, PCA analysis was also performed, to validate the significant differences in chemical composition between cultivars and genotypes of experimented fig fruits. Furthermore, by the virtue of PCA, it also confirmed some common characteristics of selected cultivars and genotypes, particularly in the peels of '01-1N-58', genotype which was found to have a higher level of polyphenols and 'Sarilop' cultivar was amongst the lowest. As Turkiye is the

most important country in terms of the production of figs, ‘Sarilop’ and ‘Bursa Siyahi’ are the most commonly grown fig cultivars in the country. However, in our study, the ‘Sarilop’ cultivar had the least PCs and antioxidant activity detected among all the experimented samples. Therefore, based on the results, the peels of ‘01-1N-58’ genotype and the peels of ‘Sarilop’ cultivar were selected for transcriptomic analysis.

For this, RNA-Seq analysis was performed on the peels of the dark-purple ‘01-1N-58’ genotype and greenish-yellowish ‘Sarilop’ cultivar using Illumina NovaSeq 6000™. Then differentially expressed genes (DEGs) among samples were determined, and based on these genes, gene ontology (GO) analysis was performed. In total, 39,430,05 (9.3%) unigenes and 38,325,911 (90.7%) duplicate genes were obtained with an average size of 5 M in the "01-1N-58’ genotype. The "Sarilop" cultivar acquired 43,348 unique genes and 44,608,828 duplicate genes with an average size of 4.7 million. A total of 21102 differentially expressed genes were observed, and 3542 up-regulated and 3164 down-regulated genes in the libraries of two fig samples were identified. qRT-PCR analysis was also conducted to validate the results of the expression of genes in RNA-seq. Four genes *CHI*, *CHS*, *PAL* and *F3’H* showed higher expression level in the peels of dark-purple O1-1N-58 genotype and depicted lower expression levels in the greenish-yellow Sarilop cultivar. Most significantly, our study indicated that the rich bioactive compounds in peels and high variability among eight fig cultivars and genotypes might allow better use of various fig fruit resources when consumed with dark-colored peels for maximum health benefits.

GENİŞLETİLMİŞ ÖZET

İncir (*Ficus carica* L.), Moraceae familyasından çiçekli bir bitki türüdür. Akdeniz Bölgesine ve Batı Asya'ya özgü olup dünyanın birçok yerinde önemli bir besin kaynağı olan meyvesi için binlerce yıldır yetiştirilmektedir. İncir, ev bahçeleri için popüler bir bitkidir ve aynı zamanda meyvesi için ticari olarak yetiştirilir. Birçok ülkenin kültüründe ve mutfaklarında önemli bir rol oynamıştır. Akdeniz iklimine iyi adapte olan incir ağaçları, Türkiye gibi ülkelerde uzun bir yetiştiricilik geçmişine sahiptir. İncir, taze veya kuru olarak tüketilen, çeşitli yemeklerde, tatlılarda kullanılan lezzetli meyveleri nedeniyle Türkiye'de yaygın olarak yetiştirilmektedir. İncir Türkiye'de önemli bir besin kaynağı olmasının yanı sıra kültürel ve ekonomik öneme de sahiptir. Genellikle ev ve meyve bahçelerinde yetiştirilir, meyveler bazı küçük çiftçiler için önemli bir gelir kaynağıdır. Ayrıca incir, Türkiye için önemli bir ihracat ürünüdür ve ülke, dünyanın en büyük incir üreticisi ve ihracatçılarından biridir.

İncir içerdiği önemli miktardaki fenolik bileşikler, şekerler ve antioksidanlar nedeniyle insanlarda kalp ve metabolik hastalıkları, mikrobiyal enfeksiyonları, obeziteyi, diyabeti ve kanseri önlemede önemli olduğu düşünülmektedir. Bu fenolik bileşikler antioksidan kapasiteleriyle de ünlüdür, özellikle antosiyaninler, meyveler, sebzeler ve tahıllar dahil olmak üzere çok çeşitli bitki bazlı gıdalarda bulunan bir pigment türüdür. Antosiyaninler birçok besinin kırmızı, mavi ve mor renklerinden sorumludurlar ve flavonoidler olarak bilinen bileşikler sınıfının bir parçasıdır ve potansiyel sağlık yararları nedeniyle çok sayıda çalışmanın konusu olmuştur.

Antosiyaninlerin potansiyel sağlık yararları, vücut hücrelerini serbest radikallerin neden olduğu hasarlardan korumaya yardımcı olabilecekleri anlamına gelen güçlü antioksidanları içerir. Kan damarlarının işlevini iyileştirerek, kan basıncını düşürerek ve kolesterol seviyelerini düşürerek kalp hastalığı riskini azaltmaya yardımcı olabilir.

Antosiyaninler hafıza üzerinde olumlu bir etkiye sahiptir. Diyabetli kişilerde insülin duyarlılığını ve kan şekeri kontrolünü iyileştirmeye yardımcı olabilir. Ayrıca, meyve kabuklarında lifler, antioksidanlar, mineraller ve vitaminler gibi birçok temel besin bulunur.

Antosiyaninlerin oluşumu ve meyve kabuğundaki klorofilin bozulması, genellikle meyve olgunlaşması, şeker birikimi ve diğer çoklu kalite özelliklerinin oluşumu ile eş zamanlı olarak ikinci hızlı büyüme aşamasında başlar. Antosiyaninden sonra şeker, bir diğer önemli meyve kalite özelliğidir. Çok sayıda genin ifadesini senkronize ederler ve polifenollerin, organik ve amino asitlerin, aroma bileşiklerinin yanı sıra pigmentlerin sentezinden sorumludurlar.

Yüksek Performanslı Sıvı Kromatografisi-Kütle Spektrometresi (HPLC-MS/MS) analizleri gıda ve bitki ekstraklarını kalite açısından değerlendirmek için kapsamlı bir yöntemdir. Türkiye dünyanın en büyük incir üreticisi olduğundan ve bu meyve beslenmenin önemli bir parçası olarak kabul edilmektedir. Genetik çeşitliliğin fazlalığı ve zengin tıbbi değerlerine rağmen, yerel incir genotipleri göz ardı edilmiş ve beslenme kaliteleri ile ilişkili biyokimyasal

karakterizasyonları açısından yeterince incelenmemiştir. Bu çalışmada, sekiz farklı incir genotipinin kabuk ve meyve etindeki biyoaktif içeriklerin karakterize edilmesi ve belirlemesi amaçlanmıştır.

Toplam dört incir (*F.carica* L.) çeşidi 'Sarılop' (yeşilimsi sarı), 'Yeşil Bozova' (yeşilimsi-açık-mor), 'Bursa Siyahi' (koyu-mor), 'İzmir Bardakçı' (sarımsı yeşil) ve dört incir genotipi '01-1N-58' (koyu-mor), '31-N-05' (koyu-mor), '01-1M-02' (yeşilimsi-mor) ve '01-1N-51' (koyu-mor) toplam fenolik bileşimler, şekerler ve toplam antioksidan kapasite açısından taranmıştır. Seçilen genotipler, meyve olgunlaşma aşamasında toplanmış ve HPLC-MS/MS yöntemiyle analiz edilmiştir. Meyveler Ağustos ve Eylül 2021'de Çukurova Üniversitesi (37°01'46.6"K-35°21'46.6"E) İncir Genetik Koleksiyonu bahçesinden toplanarak doğrudan bir buz kutusuna alınmıştır. Her genotipten en az 20 sağlıklı meyve, renklerine ve ekonomik önemlerine göre seçilmiş, toplanan örnekler Çukurova Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü Moleküler Islah Laboratuvarına getirilmiştir. Meyveler %70 etanol ile temizlendikten sonra bistüri yardımıyla kabukları soyularak, kabuk posadan ayrılmıştır. Meyve kabuğu ve meyve eti örnekleri hızlıca alüminyum folyoya sarılıp sıvı azota daldırılmış ve sonraki deneysel çalışmalar için -80°C'de muhafaza edilmiştir.

Birinci aşamada renkli-antosiyaninler ve renksiz fenolik bileşiklerin tespiti için çalışma HPLC-MS/MS yapılmıştır. Toplamda 7 antosiyanin (Cyanidin-3, 5-O-diglucoside, (Epi) catechin-(4-8)-cyanidin-3-rutinoside, (Epi) catechin-(4-8)-cyanidin-) dahil 31 fenolik bileşik 3-rutinosid, Cyanidin-3-glucoside, Cyanidin-3-O-rutinoside, Pelargonidin 3-0-rutinoside ve Cyanidin-3-malonylglucoside) ve 24 renksiz fenolik tespit edilmiştir.

Antosiyanin içeriği bakımında hem kabukta hem de meyve etinde genotipler arasında önemli farklılıklar bulunmuştur. Antosiyaninlerin kabukta maksimum ortalama değeri '01-1N-58' (koyu mor) genotipinde 151,25 mg/100 g DW olarak kaydedilirken, 'Sarılop çeşidinde (yeşil-sarımsı) minimum ortalama değer 0,23 mg/100 g DW olarak belirlenmiştir. Aynı şekilde, meyve etindeki antosiyanin değerlerinin maksimum miktarı '01-1N-58' genotipinde 31,57 mg/100 g DW olarak saptanırken, 'Sarilop' çeşidinde minimum ortalama değer 4,86 mg/100 g DW olarak bulunmuştur. Tanımlanan antosiyaninler arasında hem kabuklarda hem de meyve etinde siyanidin türevlerinin yüzdesi yüksek bulunmuştur.

Renksiz fenoliklerin miktarı (01-1N-58') genotipinde daha yüksek (1063,91 mg GAE/100 g DW) hesaplanırken, kabuklarda minimum ortalama değer 582,17 mg GAE/100 g DW olarak bulunmuştur (01-1N-51). Ayrıca çalışmamızda belirlenen renksiz fenolikler arasında klorojenik asit (CA), sekiz incir çeşidi ve genotipinin kabuklarında baskın bileşik olarak bulunmuş olup, toplam ortalama değerleri 91.13-524.74 mg GAE/100 g DW arasında değişmektedir. Benzer şekilde en yüksek değer (Sarilop) çeşidinde 780,90 mg GAE/100 g DW olarak hesaplanmış, en düşük renksiz fenolik miktarı ise incir genotipinde (31- N-05) saptanmıştır. Klorojenik asit (CA), renksiz fenoliklerin meyve kabuklarına benzer şekilde, sekiz incir genotipinin meyve etinde baskın

bileşik olarak bulunmuş olup, toplam ortalama değerleri 16.55-363.08 mg GAE/100 g DW arasında değişmektedir.

Çalışmanın ikinci aşamasında, sekiz incir genotiplerinin kabuk ve meyve etinde şeker tespiti için HPLC çalışması yapılmıştır. Kabuktaki şekerlerin maksimum ortalama değeri 'İzmir Bardakçı' (sarımsı yeşil) çeşidinde 504,64 g/ kg-1 DW olarak kaydedilirken, 'Bursa Siyahi' çeşidinde minimum ortalama değeri 380,25 g/ kg-1 DW olarak bulunmuştur. Aynı şekilde meyve etindeki toplam şeker ortalama değeri en yüksek 546,94 g/ kg-1 DW olarak Bursa Siyahi çeşidinde, en düşük ortalama değer ise 493,45 g/ kg-1 DW ile İzmir Bardakçı çeşidinde bulunmuştur. Toplam şekerler arasında glikoz, fruktoz ve sukroz ile birlikte meyve kabuğunda en yüksek oranda bulunan şekerlerdir. Glikoz ortalama değeri 183,24–238,79 g/ kg-1 DW olarak en fazla bulunan şekerler olarak belirlenmiştir. Onu sırasıyla 173,52–228,81 g/ kg-1 DW değeri ile fruktoz ve 23,50–80,62 g/ kg-1 DW ile sükroz izlemiştir. İncir genotiplerinin meyve etinde toplam şekerler içinde fruktoz ortalama değeri 225,42-270,33 g/ kg-1 DW ile baskın şeker, bunu 217,53-250,16 g/ kg-1 DW değeri ile glukoz takip etmiş ve sükroz miktarı ise 28,07-58,48 g/ kg-1 DW bulunmuştur.

Çalışmanın üçüncü aşamasında, sekiz genotipinin hem kabukları hem de meyve etinde antioksidan aktivitelerini değerlendirmek için iki analiz 2, 2'-difenil-1-pikrilhidrazil radikali (DPPH) ve 2, 2-azinobis 3-etilbenzotiazolin-6-sülfonik asit (ABTS) yapılmıştır.

'01-1N-58' incir genotipi DPPH ile ölçülen en yüksek antioksidan kapasitesine (28,08 mM Trolox /gdw) sahipken, '31-N-05' genotipi en yüksek ABTS değerine (127,34 mM Trolox /gdw) sahip olmuştur. Buna karşılık, 'Sarılop' çeşidi, sırasıyla DPPH (7,84 mM Trolox /gdw) ve ABTS (43,81 mM Trolox /gdw) ile ölçülen en düşük antioksidan kapasiteye sahip olmuştur. Meyve etinde ise, '01-1N-58 genotipi sırasıyla DPPH (9.41 mM Trolox /gdw) ve ABTS (50.94 mM Trolox /gdw) ile ölçülen en yüksek antioksidan kapasiteye sahip olmuş, '31-N-05' genotipi, sırasıyla DPPH (4.55 mM Trolox /gdw) ve ABTS (28.45mM Trolox /gdw) ile ölçülen en düşük antioksidan kapasiteye sahip olmuştur.

Ek olarak, tez kapsamında çalışılan incir genotipleri arasındaki kimyasal bileşimdeki önemli farklılıkları doğrulamak için PCA analizi de yapılmıştır. Ayrıca, PCA sayesinde, özellikle '01-1N-58' kabuklarının daha yüksek düzeyde polifenollere sahip olduğu ve 'Sarilop' çeşidinin de en düşükler arasında olduğu saptanmıştır.

Türkiye incir üretimi açısından en önemli ülke olduğundan, ülkede en çok yetiştirilen incir çeşitleri 'Sarilop' ve 'Bursa Siyahi'dir. Bununla birlikte, çalışmamızda, 'Sarilop' çeşidi, denenen tüm örnekler arasında en az PC'ye ve antioksidan aktiviteye sahip bulunmuştur. Bu nedenle, sonuçlara göre transkriptomik analiz için '01-1N-58' ve 'Sarilop' çeşitleri seçilmiştir.

Bunun için Illumina NovaSeq 6000™ sekanslama cihazı kullanılarak mor renkli '01-1N-58' incir genotipi ve ‘Sarılop' çeşidinin kabuklarında RNA-Seq analizi yapılmıştır. Daha sonra çeşitler arasında diferansiyel olarak eksprese edilen genler (DEG'ler) belirlenmiş ve bu genlere

dayanarak gen ontolojisi (GO) analizleri yapılmıştır. "01-1N-58' genotipinde ortalama 5 M büyüklüğünde toplam 39.430,05 (%9,3) unigen ve 38.325.911 (%90,7) gen elde edilmiştir. "Sarılop" çeşidinde 43.348 özgün gen ve ortalama boyutu 4,7 milyon olan 44.608.828 adet kopya genler elde edilmiştir.

Toplam 21.102 diferansiyel eksprese edilmiş gen belirlenmiş ve iki incir genotipinin kütüphanelerinde 3542 up regüle ve 3164 down regüle genler tanımlanmıştır. Ayrıca RNA-seq analizleri gen ekspresyonunun sonuçlarını doğrulamak için qRT-PCR analizleri yapılmıştır.

qRT-PCR analizlerinde kullanılan 4 gen (*CHI*, *CHS*, *PAL* ve *F3'H*) koyu mor 01-1N-58 genotipinin kabuklarında daha yüksek ekspresyon seviyesi gösterirken, yeşilimsi-sarı Sarilop çeşidinde daha düşük ekspresyon seviyeleri göstermiştir.

Sonuç olarak; çalışmamız sekiz incir çeşit ve genotipinin kabuğundaki biyoaktif bileşiklerin çeşitliliğinin yüksek olduğunu göstermiştir. Koyu renkli meyvelerin sağlık açısından önemli olduğu bir kez daha ortaya koyulmuş, incir genetik kaynaklarının bu bağlamda değerlendirilmesi daha etkin kullanılması açısından önemli olabileceği vurgulanmıştır. Transkriptom analizleri sonucunda antosiyanin ile ilişkili genlerin belirlenmesine yönelik önemli bilgiler elde edilmiş olup, bu verilerin antosiyanin metabolizmasında etkili genlerin tanımlanması ve fonksiyonel analizleri için gelecekteki çalışmalara ışık tutacağı düşünülmektedir.

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SYMBOLS AND ABBREVIATIONS

%	: Percentage
°C	: Degree Celsius
µg	: Microgram
FAO STAT	: Food and Agriculture Organization Corporate Statistical Database
PCs	: Phenolic compounds
DW	: Dry weight
CY	: Cyanidine
ANOVA	: Analysis of Variance
mg	: Milligram
PG	: Pelargonidin
Cy-3 RUTH	: Cyanidine 3-Rutinoside
FW	: Fresh weight
CTAB	: Cetyltrimethylammonium bromide
TAC	: Total antioxidant capacity
DPPH	: 2, 2-diphenyl-1-picrylhydrazyl
FRAP	: Ferric reducing antioxidant power
ORAC	: Oxygen radical absorption capacity
TRAP	: Total peroxyl radical trapping antioxidant parameter
DNA	: Deoxyribonucleic acid
dNTP	: Deoxyribonucleotide triphosphate
ABTS	: 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid
HPLC	: High-performance liquid chromatography
HPLC-MS/MS	: Liquid chromatography–mass spectrometry
NGS	: Next-generation sequencing
g	: Gram
PAGE	: Polyacrylamide gel electrophoresis
CR	: Commercial ripe
TR	: Tree ripe
UFGT	: Anthocyanidin 3-O-glucosyltransferase
GC-MS	: Gas chromatography–mass spectrometry
GAE	: Gallic acid equivalents
PCR	: Polymerase chain reaction
RP-LC	: Reversed-phase liquid chromatography

MeOH : Methanol

ISSR : Inter-simple sequence repeats

SNPs : Single nucleotide polymorphisms

RNA-Seq : RNA sequencing

LSD : Least significant difference

bHLH : Basic helix–loop–helix

H_2SO_4 : Sulfuric acid

PCA : Principal component analysis

MAS : Marker-assisted selection

Max : Maximum

EDTA : Ethylenediaminetetraacetic acid

Min : Minimum

PVP : Polyvinylpyrrolidone

SDS : Sodium dodecyl sulfate

CIA : Chloroform isoamyl alcohol

LiCl : Lithium Chloride

EtOH : Ethanol

NaCl : Sodium chloride

DEPC : Diethylpyrocarbonate

NaOAc : Sodium Acetate

RPM : Revolutions per minute

ddH2O : Double distilled water

UV : Ultraviolet

cDNA : complementary DNA

RT-PCR : Reverse transcription polymerase chain reaction

GBS : Genotyping-by-sequencing

RNA : Ribonucleic acid

dNTPs : Deoxynucleoside triphosphate

H_2O : Water

$MgCl_2$: Magnesium chloride

Rt : Retention time

CA : Chlorogenic acid

GA : Gallic acid

SSR : Simple sequence repeats

SD : Standard deviation

Taq : *Thermus aquaticus*

µl : Microliter

1. INTRODUCTION

1.1. Background

Figs (*Ficus carica* L.) are members of the Moraceae family. It is one of the first species that humans have domesticated and has long remained a staple diet in the Mediterranean region (Arvaniti et al., 2019). Furthermore, the fig was first cultivated in the Eastern Mediterranean and later moved to the Western Mediterranean region (Veberic et al., 2016). It is distinguished from other members of the genus by having milky latex throughout all parenchymatous tissue, unisexual flowers, anatropous ovules, and aggregated drupes or achenes (Barolo et al., 2014). As far as their worldwide production is concerned, fig production was reported as 1264943 tonnes (FAOSTAT, 2022). Among the worldwide production, Turkiye has been dominating the total world's fig production and is the leading producer of both fresh and dried figs with a production of approximately 320000 tonnes, followed by Egypt with 201212 tonnes and Morocco with 144246 tonnes, respectively (FAOSTAT, 2022). Therefore, *F. carica* L. has a special place in Turkish cuisine and has maintained a distinctive position in the global market with exceptional qualities such as soft texture, attractive colors, pleasant aroma and unique flavors as well (Arpacı et al., 2018) See (Figure 1.1.).



Figure 1.1. Some of the fig's varieties sold in the open markets of Adana, Turkiye

Fruits are one of nature's greatest gifts because they contain a variety of vital nutrients for our body's system maintenance and health. Among the fruits, figs are a significant source of phenolic compounds, minerals, proteins, and fibers that contributes to the color and flavor of fruit (Solomon, 2006). Moreover, they also contain a significant amount of phenolic compounds (PCs), sugars and antioxidants which not only determine the quality of fruits but are equally important for preventing heart and metabolic diseases, microbial infections, obesity, diabetes and cancer in humans (Alibabic et al., 2018). Additionally, the composition of nutrients in dried figs makes them

a wonderful source of minerals and vitamins, with 100g servings of the following: iron, 30%; calcium, 15.8%; potassium, 14%; thiamine (B1) 7.1%; and riboflavin (B2) of 6.2%. Although figs are typically eaten fresh, they can also be eaten dried. Furthermore, when the skin is cut from the branches, leaves, and fruits, gummy exude sap is released that contains a variety of compounds, proteinases and furocoumarins. The oxidative stress effect of coumarins is impaired by enzymes because they have the potential to irritate (Bonamonte et al., 2010). These substances, along with their variants, can also be utilized to produce beneficial medications (Bonamonte et al., 2010).

1.2. Phenolic compounds

Phenolic compounds are valuable secondary metabolites found in plants and perform a variety of biological functions. Phenolics have also a positive impact on human health due to their powerful antioxidant properties (Mahmoudi et al., 2016). They are vital for the prevention of pathological conditions associated with cancer, heart disease, neurodegenerative disorders, vision problems and asthma, as well as in boosting the immune system (Nakilcio et al., 2013). They have also been known to possess antimicrobial activity against several pathogens (Megdiche-Ksouri et al., 2015). Additionally, due to the rise in antibiotic resistance, there is growing interest in using medicinal herbs as a substitute for traditional drugs, particularly for the treatment of microbial agents (Tavares et al., 2008). Therefore, it has become essential to look for new antibacterial agents like phenolic compounds.

1.2.1. Phenolics in fig fruits

Like plants, phenolics are widely present in fruits as well, where they serve a specific purpose and are crucial for organoleptic assessment. Phenolic compounds from plant sources have been of great concern in scientific research in recent years because of their numerous positive effects on human health, primarily due to their general antioxidant profile (Kojic et al., 2011). Generally, the peels of the fruit contain the highest level of total phenolics. These phenolic compounds are generally present in the peel of the fruit, which is responsible for multiple and diverse colors such as purple-blue-red (Mustafa et al., 2022). Moreover, many nutrients are also found in fruit peels including fibers, antioxidants, minerals and vitamins. As far as the peel of fig fruit is concerned the fruit peel of fig accessions ranges in color from dark black to green-yellow, and they display an appealing assortment of unique colors. Studies claimed that by-products made of fig pulp and peels can be used as food additives. Peel extract can be used as a colorant due to its high anthocyanin content and adding natural purple colors while also showing intriguing antimicrobial and antioxidant properties (Zhao et al., 2020). Moreover, the polyphenol content of black fig is significantly higher than that of green fig (Alessandra and Piga, 2008).

Previous studies in *F.carica* L. confirmed the multiple anthocyanidin pigments present in the fig skin including cyanidin, cyanidin-3-O-rutinoside, Cyanidin-3-5-O-diglucoside, cyanidin-3-

glucoside, cyanidin-O-malonyl-hexoside, pelargonidin, delphinidin, malvidin, petunidin, and peonidin (Khoo et al., 2017). The identified substances come from a variety of chemical families, including flavonoids and phenolic acids. Studies on chemical composition have revealed the chemical structures of the primary compounds present in fig fruits and variants (Lugasi et al., 2002). The type of compounds varies based on the constituents of the fruit, maturity, variety, and region of the cultivar. The main individual phenolic compound in whole figs was found to be quercetin-3-O-rutioside, which was followed by polymeric procyanidins and cyanidin-3-0-rutinoside-quercentin-3-glucoside. The most prevalent bioactive component in the peel was cyanidin-3-rutinoside. The two main substances identified in fig pulp were epicatechin and cyanidin-3-rutinoside (Teruel-Andreu et al., 2021). Other important phenolic compounds present in fig whole fruit, peel and pulp are listed in (Table 1.1).

Table 1.1. Phenolic compounds identified in peels and pulps of *F. carica* L.

Plant Part used	Chemical Compound Identified	Min. Value (mg g-1DW)	Max. Value (mg g-1DW)	References
Whole fig fruit	Catechin, Rutin, Chlorogenic acid, Gallic acid, Ellagic acid, Syringic acid, Epicatechin, Quercetin 3-glucoside, Cyanidin-3-O-diglucoside, Keampferol-3-glucoside, Apigenin-C-hexoside-pentodiside	0.2060 0.0489 *0.0880* *0.0010* *0.0020* 0.0003 *0.0900* *0.0041* *0.0000* *0.0060* *0.0050*	0.9570 0.2870 *1.2450* *0.0038* *0.0008* *0.0007* *0.4310* *1.4020* *0.0190* *0.3050* *0.2430*	(Wojdyło et al., 2016; Veberic, et al., 2016; Veberic, et al., 2008).
Fig fruit peels	Catechin, Cyanidin-3,5-diglucoside, Epicatechin, Chlorogenic acid, Ellagic acid, Quercetin, Keampferol-rutinoside	0.0220 *0.0020* *0.0009* *0.0200* *0.0150* *0.0009* 0.0020	0.2060 *0.0052* *0.0239* *0.0580* *0.0330* *0.595* 0.0070	(Pereira, et al., 2017; Dueñas et al., 2008; Vallejo et al., 2012).
Fig fruit pulps	Catechin, Chlorogenic acid, Epicatechin, Cyanidin-3,5-diglucoside, Cyanidin-3-O-diglucoside, Cyanidin-33-diglucoside, Cyanidin-3-rutinoside, Ellagic acid, Quercetinrutinoside.	0.140 0.0010 *0.0140* 0.0001 0.0001 0.0005 0.0100 0.0070 0.0040	0.0670 *0.0130* 0.1330 0.0006 0.0083 0.0022 0.0950 0.0140 0.0170	*(*Pereira, et al., 2015; Dueñas et al., 2008; Vallejo et al., 2012).

1.3. Anthocyanins

Anthocyanins are water-soluble phenolic compounds found in a wide array of plants and accountable for a broad range of colors extending from colorless to purple. They are mostly found in the skin of fruits like apples, pears, grapes, blackberries, figs and plums. Even though they have few chromophore groups, over 540 natural anthocyanidic pigments have been reported in them (Vargas et al., 2013). Anthocyanins are glucosides, which are flavonoid derivatives of the phenylpropanoid pathway. Therefore, fruits containing anthocyanins are not only aesthetically pleasing but also considered an attractive attribute of the fruit quality. Anthocyanin intake for every person has been predicted to be approximately 200 mg daily. The high concentrations of phenolic compounds, especially anthocyanins, in *Ficus carica* L. are largely responsible for the plant's beneficial effects on health. Depending on the color of the fruit's skin, the fig accessions have different phenolic, anthocyanin and antioxidant profiles. Previous research studies revealed that the fruit's color directly influences its quality and the preference of the consumer (Wang et al., 2017). Fifteen anthocyanin pigments have been identified, the majority of which contained the Cyanidine (CY) pelargonidin as an aglycone; certain derivatives (PG) were also discovered. Other noteworthy compounds in the anthocyanidiques fig pigments composition include 5-carboxypyranocyanidin-3-rutinoside and cyanidine (Vinson et al., 2005). According to Solomon et al. (2006), there has been an increase in polyphenols and anthocyanins, particularly in fig fruits, along with antioxidant activity. Along with that, four anthocyanins were described in the figs among which cyanidine 3-rhamnoglucoside (Cy-3 RUTH) accounts for 75% of the anthocyanins in the fig, while the other pigments include 11% of diglucoside secoisolariciresinol, 11% of cyanidine 3-glucoside and 3% of three pelargonidin rhamnoglucoside (Solomon et al, 2006). Solomon et al. (2006) also analyzed the total anthocyanins, polyphenols, and flavonoids present in the pulp and film of commercial fig varieties with different colors (black, red, yellow, and green). Previous research studies in *F.carica* L. confirmed the presence of multiple anthocyanidin pigments in the fig peels including cyanidin, cyanidin-3-O-rutinoside, cyanidin-3-5-O-diglucoside, cyanidin-3-glucoside, cyanidin-O-malonyl-hexoside, pelargonidin, delphinidin, malvidin, petunidin, and peonidin (Dueñas et al., 2008; Khoo et al., 2017; Li et al., 2021; Solomon et al., 2006; Wang et al., 2017).

Anthocyanins with joint structure enable electron dissociation, thus developing a highly sustainable radical scavenging product with antioxidative properties (Kahkonen et al., 2003). The increased levels of phenolics and anthocyanins in strawberries, blueberries, figs and blackberries contribute to their high antioxidant capacities (Rababah et al., 2005). Figs variety named Mission was explored to possess the highest anthocyanin content of the six varieties studied by Solomon et al. (2006) with 11.0 (1.0 mg/100 g of FW). The fresh figs' total antioxidant capacity (TAC) and polyphenol and anthocyanin content are closely correlated (Yang et al., 2009).

1.4. Sugar content

Following anthocyanin, sugar is another significant fruit quality trait. They synchronize the expression of numerous genes and are responsible for the synthesis of polyphenols, organic and amino acids, aroma compounds, as well as pigments (Akšić et al., 2019). Typically, a major biochemical component of fruit is sugar, and the quantity of sugar directly affects the fruit's quality. Figs are greatly valued for their color, taste and size and contain a lot of minerals, vitamins and dietary fibers. Figs have high levels of sugar content that affect their quality. Understanding the enzymes involved in sugar metabolism is crucial. There have been numerous attempts to enhance sugar levels, which are influenced by a variety of influences during fruit development. Soluble sugars are indeed an important factor in evaluating fruit quality, therefore, research on fruit sugar accumulation will offer fundamental knowledge about fruit quality. One of the important quality characteristics is known to be the amount of sugar and its fractions.

Sugar content is a crucial determinant of fruit quality and is closely related to ripeness in the majority of fruits. Ripe fruits have more sugar than those that are still unripe. Few studies have also been conducted on the identification and determination of total sugar contents in *F.carica* L. The major sugars reported in the previous studies are sucrose, fructose and glucose, whereas sugars identified in trace amounts are lactose, ribose, arabinose, maltose and xylose (Pereira et al., 2015). Representative fruit samples from two different regions that are known to produce fig crops were examined for sugars. Glucose (6.30 g/100 g fresh weight) and fructose (5.10 g/100 g fresh weight) were the predominate sugars found in Tunisian figs (Ersoy et al., 2007). Fresh fig fruits were analyzed for the presence of the most prevalent sugars it was recommended that the sugar content found in fig fruit can be considered a healthy alternative to sweets as figs also possess antioxidant properties (Veberic et al., 2008).

1.5. Antioxidant activities of *Ficus carica L.*

Natural antioxidants, which are primarily phenolics are present in all plant materials, including fruits, green leafy vegetables, bark, seeds, and rhizome (Nencini et al., 2011). Plant phenols and polyphenols, which have the potential to function as antioxidants, are crucial in preventing several pathological ailments, including cancer, cardiovascular diseases and neurodegenerative diseases that are thought to be linked to oxidative stress (Losso et al., 2007). The antioxidant capacity of human plasma is markedly increased by the consumption of dried figs.

Fig fruits are low in fat and cholesterol and high in amino acids. They contain a variety of biologically active compounds in abundance, such as phenolics, which are largely responsible for their antioxidant activity. The genotypes and fig cultivars have a noteworthy impact on the concentration levels of these compounds. Recent research has also shown that fig genotypes and cultivars can have a significant impact on other physicochemical properties like color, fresh and dry matter, pH, total dissolved solids, sugars, fibers, acids and anthocyanins (Ercisli et al., 2012).

Halverson et al., (2002) examined total antioxidant activity in a variety of dietary plants and discovered that Turkish Smyrna figs (honey color) seemed to have a higher antioxidant potential than papaya, apple, mango and banana, but lower than pomegranate, grape, or plum (Halverson et al., 2002).

The Mediterranean diet seems to have a lot of naturally occurring antioxidants found in fruits like figs, which contain some of the highest polyphenol concentrations of any commonly available fruit (Solomon et al., 2006). Solomon et al. (2006) showed that the higher the polyphenol content, particularly anthocyanins, in fig fruit, the higher their antioxidant activity. Antioxidants from figs can protect plasma lipoproteins from oxidation and significantly elevate the plasma antioxidant capacity for 4 hrs after consumption (Vinson et al., 2005).

The maximum concentrations of phenolic acids, flavonoids and anthocyanins, as well as the highest antioxidant properties, were reported in fig fruits. Numerous fig accessions have yet to be compared in context to phytochemical traits like phenolic contents (colored and non-colored), sugars and antioxidant capacity. Various antioxidant methods like DPPH (2, 2′-diphenyl-1-picrylhydrazyl radical), FRAP (Ferric reducing antioxidant power), ORAC (Oxygen radical absorption capacity), TRAP (Total peroxyl radical trapping antioxidant parameter), ABTS (2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid), HORAC (Hydroxyl radical antioxidant capacity), TOSC (Total oxyradical scavenging capacity), CUPRAC (Cupric reducing antioxidant power) and the Folin- ciocalteu tests have been used for the analyses of the antioxidant activity. Whereas, by in large the ABTS assay is used to evaluate a sample's antioxidant activity based on radical cation ABTS'+ scavenging capacity. This method, we used Trolox (a water-soluble version of vitamin E) as a standard and is commonly used to assess antioxidant activity. Whereas, the DPPH radical scavenging test is also a simple and rapid method of assessing antioxidant activity by assessing the absorbance of a solution following the blend of antioxidant compositions with DPPH radicals (Baliyan et al., 2022).

This study measured the total phenolics, anthocyanin, sugar composition color profile and antioxidant potential of selected fig accessions and data can be utilized to create health-promoting fig cultivars. The HPLC fingerprint is a comprehensive method of assessment to check the quality and reliability of food and plant extracts (Mustafa, et al., 2022). As per reviewed literature, few studies have been performed and published on the bioactive content of fig genotypes. However, no comprehensive work has been carried out on the HPLC-MS/MS analysis of the total phenolic compositions, HPLC analysis of sugars and total antioxidant activity (DPPH and ABTS) analysis in both the skin and pulp of Turkish *F. carica* L.

1.6. A transcriptomic aspect of *Ficus carica L.* focusing on anthocyanins

Turkey is one of the world's most significant genetic origins of fig *(Ficus carica L.)* and it is extended to the Mediterranean region including (Greece, Tunisia, Morocco, Spain, France and Algeria). In Turkey, fig germplasm is mostly found in the Aegean region's Big Meander Valley and Small Meander Valley, but it is also found in Southeast Anatolia and Mediterranean countries (Tanriver, 2019). In recent years, next-generation sequencing (NGS) technologies have enabled researchers to conduct extensive gene-expression studies and high-throughput sequencing in fig and other model and non-model plants (Freiman et al., 2014).

Recently, several studies on the transcriptomic attributes of fig fruit maturation and ripening have been conducted and compared the predicted transcriptomes of Dottato and Horaishi which are two important cultivars, as a result around 4000 species-specific genes were found in them respectively (Zambrano et al., 2017). A comparison of transcriptomes in common fig fruit and young San Pedro type fig revealed that biosynthesis of zeatin and transduction of plant hormones signaling mechanisms are selectively constrained and ethylene formation and phytohormone signaling were reported to be overexpressed for both caprifig and common fig fruit (Ikegami et al., 2013: Chai et al., 2017). Genes that encode ethylene-response factors, fruit cellular enzymes and ascorbate oxidase were detected to be highly expressed during fig fruiting at the transcriptomic level of plants (Freiman et al., 2014). Transcriptomic evidence also indicated that the fig flesh has distinct climacteric characteristics throughout maturation. Proteins, the final gene expression product, is the final crucial executor of bio functions. The proteomic analysis could yield more accurate data on important biological processes like the ripening process and fruiting (Yu et al., 2022). In comparison to methods based on conventional 2D-polyacrylamide gel electrophoresis (PAGE), isobaric tags for absolute and relative quantitation (iTRAQ) is a quantitative technology commonly applied in proteomics studies (Kambiranda et al., 2014). It increases the precision and reliability of protein quantification and as a result, it might offer a potent tool for illuminating and speculating on the intricate and universal modifications influencing fruit ripening, quality assessment and agricultural attributes thus boosting commercial and industrial fig quality assurance and innovation. Fresh figs post-harvest handling, senescence, quality improvement, and shelf life are all greatly impacted by harvesting at various ripening stages as they are perishable fruit (Freiman et al., 2012). However, little is known about the proteomic and transcriptomic data of figs at the commercial-ripe CR and tree-ripe TR stages.

1.7. Regulation of anthocyanin biosynthesis

Fruit skin pigmentation is a distinct phase of the life cycle of fruit development in plants, primarily caused by anthocyanin pigments. The most diverse plant pigments derived from the phenylpropanoid pathway are anthocyanins. They are water-soluble polyphenols that are part of the large and diverse class of plant flavonoids. The coloration of red fruits owing to anthocyanins

is an essential part of their appearance value and thus contributes to their market value (Jaakola, 2013). In general, consumers prefer colored fruits because the color is always attributed to flavor. Color refers to a variety of physiological and biochemical changes that occur as a result of the variable expression of several developmental-level responsive genes (Senica et al., 2019). Chalcone synthase, dihydroflavonol-4-reductase, chalcone isomerase, anthocyanidin synthase, and UDP-glucose: flavonoid 3-O-glucosyltransferase are the structural genes that encode the essential enzymes in anthocyanin biosynthesis (Jaakola, 2013).

Several enzymes from the phenylpropanoid and flavonoid biosynthetic pathways catalyze the metabolism of anthocyanins (Falcone et al., 2012). Cinnamic acid 4-hydroxylase, chalcone isomerase, chalcone synthase, flavanone 3-hydroxylase and flavonoid 3′5′-hydroxylase are just a few of the enzymes acting as the initial precursors of anthocyanins and other flavonoids. The entire metabolic pathway is then used by Anthocyanidin synthase to create unstable colored anthocyanins from colorless anthocyanins (Falcone et al., 2012). Finally, flavonoid 3-O-glucosyltransferase (UFGT) converts the unstable colored anthocyanins into blue-violet, brick-red, or magenta glycosides, resulting in diverse types and amounts of substituents in the anthocyanin's ring, which determine the color hue and color depth of the anthocyanidins in different tissues and transcriptional environments (Espley et al., 2007). Color development has been studied using combined high-throughput methods in recent years. Interconnected transcriptomic and metabolomic channel evaluations in fruit and flowers have revealed several secondary metabolites with altered content, as well as the associated differentially regulated genes (Ying et al., 2019) expanding our understanding of plant color regulation. During the ripening process of a dark-colored fig, *FcANS1* gene expression was found to be sharply upregulated in the peel (Cao et al., 2016), whereas *UFGT* was found to be the essential gene for coding biosynthesis of anthocyanins in strawberries and grapes (Griesser et al., 2008). Color development has been studied using combined high-throughput methods in recent years. Comprehensive metabolomic and transcriptomic system analysis results in fruit and flowers have revealed several secondary metabolites with altered content, as well as the correlating expression levels of genes Lou et al. (2014), expanding our understanding of plant color regulation.

Several transcription factors regulate the expression of the structural genes that produce anthocyanins (TFs). The conserved domains found in TFs are used to classify them roughly into the MYB, bHLH, WD40, MADS-box, and bZIP families, respectively (Hichri et al., 2010). Among them, MYB family has been the choice of most researchers. The R2R3-MYB TF has received most of the attention in studies on the MYB TF family's role in the control of anthocyanin biosynthesis, and research on how this TF affects anthocyanin levels in fruit trees was initiated much later. Espley et al. (2007), discovered the *MdMYB1* gene for light-induced anthocyanin synthesis in apples. They also discovered the *MdMYB10* and *MdMYB110* genes, which control the pulp's anthocyanin biosynthesis. In plants like the strawberry, sweet cherry, peach, pear and

pomegranate, the homologous genes were successively isolated after the detachment and functional verification of the key TFs, MdMYB1 and MdMYB10, of anthocyanin in the apple. Inhibitors of anthocyanin synthesis include *FaMYB1* in strawberries, *VvMYBC* in grapes and *AtMYBL2* in Arabidopsis. R2R3-MYB regulation could be split into activation and inhibition (Wang et al., 2021).

1.8. Key genes for anthocyanin biosynthesis

Green, yellow, and red fig fruits accumulate varying anthocyanin content in their peel extract and drupelets within the syconia (Khadivi et al., 2018). The red color is determined primarily by the species and intensity of anthocyanins. Previous research found cyanidin-3-O-glucoside, pelargonidin-3-glucoside, cyanidin-3,5-diglucoside and cyanidin-3-rutinoside in purple fig fruits (Ercisli et al., 2012), while cyanidin was the major anthocyanin found in peels of the red cultivar 'Brown Turkey' (Sun et al., 2013). Anthocyanin biosynthetic pathways in vascular plants have been extensively researched (Tanaka et al., 2008). A sequence of enzymes catalyzes the biosynthesis of anthocyanin. L-phenylalanine is the first amino acid in the first section. Cinnamic acid, p-coumaryl CoA, and 4-coumaryl CoA are formed sequentially by phenylalanine ammonium synthases, 4-coumaryl CoA ligase and cinnamic acid 4-hydroxylase. These steps are part of the biosynthetic pathways, and the products are the biochemical forerunner of flavonoids, lignins, and other compounds. Naringenin is produced by the catalysis of the enzymes chalcone synthase and chalcone isomerase, and it is later transformed into dihydroflavonols by the flavanone 3'-hydroxylase and flavanone 3-hydroxylase enzymes.

1.9. Objectives of the study

1.9.1. General objective

As the world's largest producer of figs, this fruit is recognized as a vital part of the Turkish diet. Despite the great diversity, local figs have remained poorly examined for biochemical fingerprints associated with their nutritional quality, primarily due to the high cost of classic methods. Consequently, the present study was intended to characterize and determine the qualitative and quantitative composition of the differences in the bioactive content in the peels and pulps of eight distinct Turkish commercial fig varieties and selected genotypes during their tree ripening stage, using High-Performance Liquid Chromatography descriptive data.

1.9.2. Specific objectives

- To investigate the mechanism of anthocyanin biosynthetic genes and determine their regulatory roles in fig coloration.
- To facilitate the genetic improvement of high-anthocyanin fig fruit cultivars.

2. LITERATURE REVIEW

Bioactive compounds in plants are extremely important in the field of nutrition for providing basic nutrients, preventing diseases and improving life quality across the globe (Namsa et al., 2011). Plant-based medicines are being used for centuries ago, however, standardized assessment is required to explore the potential benefits (Chandel et al., 2011; Kaur et al., 2011; Gomez-Estrada et al., 2011). Epidemiological data showed an inverse relationship between their consumption and decreased disease frequency (Kasuli, 2011). Numerous substances found in fruits and vegetables are broadly categorized as phytochemicals and have significant effects, particularly on the prevention of disease. More organized and systematic research on these bioactive molecules is observed in recent years, which has resulted in the development of a distinct category of food called nutraceutical and functional foods. These health-promoting products are attracting the consumer's attention for their increased acceptability and level of awareness. These biologically active ingredients, which are widely present in food like spices, herbs, tea, fruits, and vegetables, exhibit significant antioxidant activity in vivo and in vitro with major health implications (Butt and Sultan, 2011).

For nearly 80% of individuals living in developing countries, traditional remedies based on medicinal plants represent the most significant source of therapeutic care (WHO, 2014). Different plant parts, such as the roots, stems, leaves, flowers, fruits and seeds accumulate phytochemicals. The outer layers of the various plant tissues frequently contain huge concentrations of several phytochemicals, including pigment molecules. According to Saxena et al. (2013), the levels differ from plant to plant based on variety, treatment and growth conditions. It was obvious that phytochemical-rich food consumption results in obtaining desirable antioxidant status and prevention of degenerative disease onset (Ancos et al., 2015). Because of its rapid therapeutic effect potential, the spread of modern health services has posed a significant threat to indigenous practices in recent years, still, medicinal plants are considered a significant source of treating various diseases in less developed countries in this era of modern pharmacology (Karunamoorthi et al., 2014).

Ficus carica L., which grows in tropical and subtropical regions, is undoubtedly an important source of foodstuffs for human nutrition (Al-Jowari et al., 2011) and a widely consumed human dietary element for being an excellent source of minerals, vitamins, and dietary fibers, all of which are correlated with health gains and endurance (Solomon et al., 2006; Oliveira et al., 2010). *Ficus* is among the most diverse medicinal plant genera, with over 750 species of woody plants, trees, and shrubs found primarily in tropical and subtropical regions around the world. Fig plant cultivation is an ideal practice in the Mediterranean and Eastern regions of Southeast Anatolia (Caliskan and Polat, 2011).

As far as their worldwide production is concerned, fig production was reported as 1264943 tonnes (FAOSTAT, 2022). Among the worldwide production, Turkiye has been dominating the total world's fig production and is the leading producer of both fresh and dried figs with a production of approximately 320000 tonnes, followed by Egypt with 201212 tonnes and Morocco with 144246 tonnes, respectively (FAOSTAT, 2022). *Ficus carica* L. originated in Asia, however, the Mediterranean basins are now the primary producers of figs (Geun-Oh et al., 2011). Fig is categorized as the significant consumable crop of the world in both dried and fresh forms (Solomon et al., 2006). Nearly 90% of harvested figs are being used in dried form (Jeong and Lachance, 2001). Figs can also act as a mild laxative, expectorant and hypotensive (Solomon et al., 2006). Dried figs are said to be packed with carbohydrates, sugar content, minerals, vitamins, organic acids and phenolics (Veberic et al., 2008).

Both fresh and dried figs are rich in fibers and polyphenols. The figs are found to be a rich source of amino acids. These are also free of fat and cholesterol (Guarrera, 2005). Phytochemical screening of *Ficus carica* L. proved it to be a rich source of flavonoids and polyphenols, as well as other compounds like arabinose, b-carotines, amyrins, b-glycosides, and b-xanthotoxol and setosterols (Jeong et al., 2009). The aqueous extract of dried *Ficus carica* L. fruits have been found to contain saponins, terpenes, flavonoids, alkaloids and coumarins (Gilani et al., 2008). Both fresh and dried fig fruits are nutrient-rich edible foods for human beings with various health benefits (IPGRI, 2003).

Countries with mild winters and dry summers are major fig producers such as Italy, Brazil, Spain, Morocco, Greece, Egypt and Turkiye. Fig fruit is generally available in dried form to make it available in all seasons. Most probably fig is the first cultivated fruit plant even before olives, wines and Grenada (Kislev et al., 2006). The world's fig production is centered in the Mediterranean regions. Figs have indeed been cultivated in this area since the Middle Ages and are among the most widely discussed fruit in the Bible (Slavin, 2006). The figs are frequently eaten raw, whether they are open or closed. The fruits have a limited shelf life after harvest (7–10 days), but under colder conditions and in an atmosphere that is higher in CO_2, the fruit can be kept for nearly about 2-4 weeks (Sozzi et al., 2005). Figs are a crucial part of the Mediterranean diet as seasonal foods (Solomon et al., 2006). Like other varieties of fruit, figs have high levels of sugar and phenolic compounds that have an impact on quality. Additionally, it contains phenolic acids, which contribute to its quality. This is significant because recent research has shown that the consumption of phenolic compounds can improve human health. The level of Phenolic content in fruit is significantly influenced by the variety of fruit and the part from where it is extracted. The crude fiber content of 5.5%, w/w and polyphenols are highly reported in fresh and dried figs (Vinson et al., 2005). Solomon et al. (2006) analyzed the total polyphenols, anthocyanins and flavonoids present in the commercial fig varieties with different colors (black, red, yellow, and green) were investigated. The skin of the fruit has provided most of the polyphenols and

antioxidant activity compared to the pulp of the fig fruit, particularly in the darker varieties. They further discovered that the more polyphenol and anthocyanins content present in figs, the higher the antioxidant activity. Antioxidants can preserve plasma lipoproteins and significantly increase oxidation and plasma antioxidant capacity after 4 hours of consumption (Vinson et al., 2005).

Ancos et al. (2015) identify a significant amount of alkaloids and little saponins in fig extract in their study, moreover, they also found antioxidants like phenols, vitamin E, carotenoids, organic acids and free radicals which are risk factors for degenerative disease development. Phenolic compounds are the secondary metabolic byproducts of the common plant which have a positive effect on plant physiological functions and human health of their ability to act as an antioxidant compound.

Figs are a high source of minerals and sugars (Vinson, 2001), particularly fructose and glucose (Al-Jowari et al., 2011). Numerous phenolic compounds, flavonoids Veberic et al. (2008) anthocyanins and fatty acids have been identified in fig fruits. However, in previous studies there is a lack of comparison among different fig accessions for phytochemical properties like total phenol, anthocyanin and sugar content were observed (Çalişkan and Polat, 2011).

Chemical characterization performed through GC-MS revealed that there are about 68 major compounds identified in fig fruits of which campesterol, oleic acid, Beta-Amyrin, and isoamyl Laurate are quite significant. Glycyrrhizine which can be used as a natural sweetener probably originates from β- amyrin is also reported in figs (Solomon et al., 2006; Oliveira et al., 2010).

These fruits are an amazing source of trace minerals, micronutrients, amino acids, raw soluble and insoluble dietary fibers and phenolic compounds (Solomon, 2006). Phenolic compounds are essential components of the fruit's quality as they contribute to enhancing the flavor and color of fruits. Kojic et al. (2011) proposed that the composition of nutrients in dried figs can become a great source of minerals and vitamins, with 100 g servings of the following: iron, 30%; calcium, 15.8%; potassium, 14%; thiamine (B1) 7.1%; and riboflavin (B2) of 6.2%. Phenolic compounds from natural sources have been of interest in scientific research in recent years because many of their positive effects were seen on human health with a primary focus on their total antioxidant activity.

According to the study by Gozlekci et al. (2011) carried out on the fig fruit of tropical and subtropical regions of the world, and they observed that *Ficus* is one of the largest genera of flowering plants in the mulberry family, with more than 800 species of trees, shrubs, vines, and epiphytes and is nature’s greatest gift for containing a huge variety of vital nutrient for maintaining good health.

Fruit peels contain a wide variety of phytochemicals, which include carotenoids, vitamins, dietary fibers, and phenolic compounds, some of which have powerful antioxidant properties. Nonetheless, there has been limited progress in screening and describing the rich variety of

phenolic compounds found in different fruit peels. Hence, Suleria et al. (2020) assess the number of phenolic compounds and antioxidant potential of twenty fruit peel samples extracted in ethanol, as well as proposed extensive identification and quantitative determination using LC-MS/MS and HPLC. 176 phenolic compounds were identified and characterized using LC-ESI-QTOF-MS/MS including 86 flavonoids, 49 phenolic acids, 25 polyphenols, 11 ligans and 5 stilbenes in twenty samples under study samples. Quantification of samples performed via HPLC-PDA showed that mango peels contain greater phenolic content, especially gallic acid and quercetin. This study highlighted the significance of fruit peels as a source of phenolic compounds and provides evidence to support the use of various phenolic-rich fruit peels as components of food, and nutraceutical products.

Nadeem et al. (2018) assess the variability in polyphenolic compounds, antioxidant properties, and different pigment levels in figs. They collect fig leaves at four stages of maturation and identify polyphenols through HPLC-DAD. Radical scavenging activity, total phenolic contents, and various pigment levels were also measured. The fig leaves contained fourteen polyphenolic compounds, according to the HPLC analysis. During maturation, significant quantitative changes were seen, especially in polyphenolic compounds. The number of pigments, total phenolic compounds, and the radical scavenging activity all changed significantly. All maturational stages of fig leaves are rich sources of polyphenolic compounds.

Çalişkan et al. (2008) determined the quality and characteristics of some fig fruit cultivars in Dortyol, Hatay province of Turkey, through the evaluation of Turkish fig cultivars including ‘Sarilop’, ‘Bursa Siyahi’, ‘Goklop’, ‘Yediveren’, ‘Yesilguz’, ‘Morguz’, ‘Sari Zeybek’, and ‘Ufak Yesil’ as well as 24 selections from large collections of Mediterranean region was also investigated. They came out with the results that Yediveren, Goklop and 31-IN-16 are proved to be the best alternative to ‘Bursa Siyahi’, which is the most preferred fresh table fig fruit cultivar, thus these cultivars gain attention for having potential for local use and export.

Likewise, a study conducted by Hssaini et al. (2020) and eleven indigenous and introduced fig cultivars grown in the Moroccan climate were examined for their morphological characters, organic acids, sugars, total phenols, flavonoids, total anthocyanins, and total anthocyanidins, antioxidant activity, and color parameters like lightness (L*), chroma (c*), and hue angle (h°). This study compared local clones to some introduced cultivars focusing on their biochemical and morphological traits. The results revealed highly significant differences between genotypes. The most empowering morphological traits were found in the cultivar "Kadota." All cultivars had slightly similar levels of reducing sugars. In general, dark-colored cultivars seemed to have higher concentrations of total phenols, flavonoids, and anthocyanins, whereas light-colored cultivars had higher concentrations of total proanthocyanidins. The highest concentrations of phenolic content and total anthocyanins were found in the local cultivars "Fassi" and "Noukali," respectively. Light-

colored figs typically had higher free radical scavenging activity (DPPH and ABTS), whereas dark-skinned cultivars typically had a higher ferric-reducing ability (FRAP).

2.1. Phenolic content

Phenolic compounds play a crucial part in fruit quality as they affect the nutritional profile, color and flavor of fruits. Veberic et al. (2008) evaluated the phenolic profile of figs by utilizing the HPLC-PDA (photo diode array detector technique) and found some important compounds such as chlorogenic acid, epicatechin, rutin, gallic acid, syringic acid and phenolics. They extract phenolics from three cultivars grown in coastal regions and analyzed that phenolics content present in fig cultivars can be compared with other fruits of the region. These cultivars included the white fruit "kofjotka" (also known as "Zuccherina"), "Črna petrovka," and the dark fruit "Miljska figa." They gathered and compared the fruit from both the first and second crops. Fruit from the second crop generally had higher phenolic content than the fruit of the first crop. The highest phenolic content observed was rutin i.e., 28.7 mg per 100 mg. This level of rutin is significantly higher in comparison to apple fruit. Further studies on phenolic content levels in dried fig fruits can make it a popular dietary constituent and confectionary alternative.

In another study, Kamiloglu et al. (2015) perform measurements of phenolic compounds, anthocyanins, flavonoids, proanthocyanins and antioxidant capacity, to investigate the impact of sun drying on these major constituents related to health and bioaccessibility present in two commercial fig cultivars that are Sarilop and Bursa siyahi with yellow and purple color respectively. Rutin and cyanidin-3-rutinoside were identified as the primary flavanol and anthocyanin in both varieties, respectively. Lower levels of total phenolics, anthocyanins, and antioxidant activity were found in both varieties after sun drying. On the other hand, yellow figs were found to have 75 and 71% higher total flavonoid and proanthocyanin contents. Phenolic compounds are food components with anticarcinogenic, antioxidative, antimutagenic, free radical scavenging, and lipid peroxidation inhibition properties. The edible fruit fig grows in the tropics and subtropics. Sarilop is a fig variety that has long been linked with horticulture in Turkey's Aegean region.

Nakilcioğlu et al. (2013) analyzed ten fresh and ten dried samples of fig cultivars found in Turkiye and determine phenolic and flavonoid content, and antioxidant activities by applying DPPH as well as qualitative and quantitative analysis of chosen samples via HPLC. HPLC was used to determine gallic acid, chlorogenic acid, syringic acid, (-)-epicatechin, rutin, and psoralen in fresh and dried figs. The main phenolic compound was identified as (-)-epicatechin. The statistical difference between different types of figs is quite significant ($P< 0.05$). It was found that fresh figs have a higher polyphenol content than dried figs.

Likewise, a study was conducted by Piga et al. (2005) on two fig varieties, one black and one white. Fresh fruits were peeled, and phenols were extracted from the peel and pulp separately.

HPLC-DAD analysis was performed on the extracts, with monitoring at four wavelengths, 280, 316, 365, and 520 for catechins, hydroxycinnamic acids, flavanols, and anthocyanins, respectively. The results revealed that phenols are almost entirely concentrated in the peel, with the black cultivar having the highest content. The peel was high in flavanols, ranging from 723 to 1450 mg/kg (fresh basis) for white and black figs, respectively. Anthocyanins, the only polyphenolic compounds found in the pulp of black figs, were found in significant amounts in the peel of the fruit. There were no catechins discovered.

Del Caro et al. (2008) performed an analytical study on phenolic content in two fig cultivars black and green. They extracted phenols from the peel and pulp of fig fruit separately. HPLC-DAD analysis was performed on the extracts, taking measurements at four wavelengths, 280, 316, 365, and 520 nm for catechins and benzoic acids, hydroxycinnamic acids, Xavonols, and anthocyanins, respectively. The results revealed that phenols are almost entirely concentrated in the peel, with the black cultivar containing the highest content. Level of Rutin levels in the peel of green and black varieties ranged from 527 to 1071 mg/kg (fresh basis), respectively. The pulp of both the green and black varieties comprised only cyanidin 3-O-rutinoside, whereas the peel of the black fig variety contained only cyanidin 3-O-glucoside. Peels of both cultivars were found to contain chlorogenic acid and cinnamic acid, but no benzoic acids or catechins were found.

Mahmoudi et al. (2016) conducted a study to ascertain the total flavonoid and phenolic contents, antimicrobial, and antioxidant properties of methanolic leaf extracts from ten varieties of Algerian figs (*Ficus carica L.)* (uniferous, biferous and caprifig tree). Folin-Ciocalteu colorimetric analysis was used to analyze phenolics extracted via Soxhlet method. The DPPH assay was used to determine the antioxidant capacity and the aluminum trichloride assay was used to determine flavonoids. The macro dilution broth method was used to calculate the minimum inhibitory concentration concentrations and minimal lethal concentrations and antimicrobial activity was investigated using the disc diffusion method. All the tested extracts have phenolic compounds, thus showing antioxidant and antibacterial properties, therefore identification and purification of phenolic compounds are highly suggested by this study.

Shahinuzzaman et al. (2021) used optimized response surface methodology in their study to maximize the extracted amount of phenolics and other bioactive compounds from *Ficus auriculate* and assess the antioxidant properties of total extracted phenolics via DPPH and ABTS assays. They concluded that at the optimum extraction conditions, the maximum yield of total phenolic content was found to be 0.94-31.65 mg GAE/g DF. The LC-ESI-MS profiling of the 18 bioactive compounds obtained from optimized extracts reveals their ability to control the antioxidant activity in *F. auriculata* fruits.

For the first time, the phenolic content and antioxidant ability of dried fruits from 17 different *Ficus carica* varieties from Tunisia's green, red, and black phenotypes were evaluated in a study conducted by Faleh et al., (2012). Analysis was performed using HPLC-DAD and every

sample displayed a comparable qualitative profile. The range of phenolics was reported as 29.18 and 55.56 mg/kg in black and red varieties, respectively. All varieties were reported to exhibit significant radical scavenging activity in the DPPH assay, however, one variety named Bayoudi was considered to be the most effective one showing IC50 values of 10.32 and 2.89 μg/ml. Phenolic composition and antiradical activity promote the consumption of fig fruit varieties for health benefits.

2.2. Sugar content

Ersoy et al. (2007) used high-performance liquid chromatography to measure the sugar content of fig fruits harvested at various stages of development and analyze changes in the major and minor sugar content of fig fruits. Galactose and fructose contents were reported to be higher than glucose and sucrose in their study. It was discovered that the concentration of sucrose was higher in the first and third fruit-growing stages than in the second. Additionally, trace amounts of ribose, xylose, arabinose, maltose, and lactose were also detected.

Sedaghat et al. (2018) studied the impact of amylase and invertase enzyme activity on sugars, starch and organic acids at developing stages such as fruit softening and senescence stages in *Ficus carica* L. cv Sabz with three replicates. The early stage of fruit development had the most stored carbohydrates and the least total sugar, whereas the senescence stage had the least stored carbohydrates and the most total sugar. Additionally, a high-performance liquid chromatograph analysis showed that fructose and glucose values significantly enhanced with fruit development. The most prevalent organic acid was malic acid, which was followed by citric acid. Invertase activity also sharply increased before the caprification stage and continued to do so until the senescence stage. They further discovered that the amylase activities in fig fruit dramatically increased up to the point of ripening and then declined slightly at the point of senescence. The current study demonstrated that fig fruit cultivars are rich in glucose, fructose, citric, and malic acids.

Likewise, five Tunisian cultivars of *Ficus carica L.* including "Bouhouli" (BHL) and "Zidi" (ZD) (dark skin figs), "Thgagli" (THG), "Bidhi" (BD), and "Khedri" (KHD) were examined by Trad et al. (2014) for their nutritional value (yellow-green skin figs). Representative fruit samples were taken from two different regions that are known to produce fig crops and were analyzed for sugar content, dietary fibers, organic acids and polyphenols. Trad et al. (2014) further determined that figs are a high-sugar fruit with a considerable dietary fiber content when compared to other types of fruit. The most intriguing figs were dark-skinned "ZD" fruit because they contained the most sugars, phenolics, organic acids and particularly the cyanidin-3-rutinoside. This cultivar was considered more suitable for fresh food consumption.

The sugar and organic acid content of fig juice from high-yielding cultivars like Colar, Florancha, To-Antonio, and Gobernador was measured by Melgarejo et al. (2001). Total sugars

such as fructose, glucose, sucrose, and maltose contents were also examined in the study. The most common sugar discovered by them was glucose, followed by fructose, sucrose and maltose concentrations were all less than 1%. Oxalic, citric, malic, tartaric, ascorbic, fumaric, and acetic acids were the most common organic acids identified. According to data from the first crop juice, the predominant acids were oxalic and citric, whereas the second crop juice had high levels of malic and tartaric acids.

Fresh fig fruit was tested by Veberic et al. (2008) in a study for the presence of the most prevalent sugars, organic acids, total phenols, and antioxidative potential. Three cultivars were investigated: "Bela petrovka" exhibited a white fruit "Crna petrovka" a dark fruit, and "Miljska figa" a dark fruit, respectively. The harvests of the figs from the summer and the fall were compared. There was no discernible difference in the fruit from the agricultural seasons. While the sugar and organic acid contents differed significantly, there were no considerable differences between the three cultivars. Figs, either fresh or dried, were recommended as a healthy alternative to sweets due to the antioxidant properties of the total phenolics and the high sugar levels.

2.2.1. Sugar transcriptomics

Ficus carica L. trees are commonly grown in the Mediterranean area, where salinity is an increasing problem in coastal areas. Mascellani et al. (2021) used Quantitative real-time PCR and quantitative 1H nuclear magnetic resonance spectroscopy to examine changes in the transcription of the primary sugar metabolism-related genes involved in the synthesis, accumulation, and transport of soluble carbohydrates in ripe fruits to better understand the impact of salinity stress. They observed an overall increase in the transcriptome of genes responsible for the transportation of soluble carbohydrates. Transcripts of alkaline-neutral and acid invertases, which are involved in the synthesis of glucose and fructose, were found to be up-regulated in ripe fruits of NaCl-stressed plants without even a change in D-glucose and D-fructose content. The significant increase in sucrose and D-sorbitol content were most likely to be caused by increased transcription of genes encoding Sucrose-Synthase and Sorbitol-Dehydrogenase.

2.3. Antioxidant activity

Sertkaya et al. (2021) conducted an antioxidant analysis study on peels and pulps of two commercially grown cultivars Sarilop and Bursa Siyahi grown in Turkiye. ABTS and DPPH analysis was carried out. Based on the results of the analysis the peels of Bursa Sayahi exhibited the total antioxidant activity as compared to the peels of Sarilop and pulps of both cultivars.

In another study conducted by Harzallah et al. (2016) fig juices from the peel, pulp, and entire fruit of three different varieties grown along the Mediterranean coastal areas of Tunisia were used to evaluate differences in phytochemical composition and antioxidant properties. In this study, the effects of the maturation stage on the amounts of phytochemical composition, including

total phenols content, total flavonoid content, and total ortho-diphenols, were assessed. Two assays were used to measure the antioxidant potential: reducing power and DPPH scavenging capacity. The results showed that different fig juice extracts had the same antioxidant capacity in both systems and at various concentrations. Black peel juice was the most effective antioxidant, with the maximum DPPH and RP activities, followed by black fig total fruit juice. According to the colorimetric results, the observed antioxidant capacities were attributed to increased total phenolic, flavonoid, and anthocyanin contents. This was the very initial study to compare the phytochemical makeup and antioxidant ability of the juices from the peels, pulps, and entire fruits of *Ficus carica L.*

To investigate the potential health-promoting constituents of fig fruits Solomon et al. (2006) measured the total polyphenols, flavonoids, antioxidant capacity and the amount and profile of anthocyanins in six commercial fig varieties (black, red, yellow, and green). Various concentrations of anthocyanins were tested using reversed-phase liquid chromatography (RP-LC), but all varieties studied had a similar profile. The major aglycon revealed during hydrolysis was cyanidin. The main anthocyanin in all fruits was identified using proton and carbon NMR as cyanidin-3-O-rhamnoglucoside (cyanidin-3-O-rutinoside; C3R). The color of fig extract was found to be highly correlated with phenolic compounds, anthocyanins, flavonoids, and antioxidant properties. Extracts from darker varieties contained more phytochemicals than extracts from lighter-colored varieties. In comparison to the fruit pulp, the fruit skins contributed most of the aforementioned phytochemicals and antioxidant activity. The Mission variety's fruits had the greatest level of phenolics, flavonoids, and anthocyanins, as well as the maximum antioxidant capacity.

Farooq et al. (2019) perform a phytochemical screening analysis on the aqueous extract of *Ficus carica* peels to assess the antioxidant activity and phytochemical makeup. They confirmed the presence of alkaloids, coumarins, flavonoids, steroids, tannins and quinones in the peel extract of *Ficus carica.* Antioxidant properties were also evaluated through DPPH assay. Various significant antioxidants, minerals and vitamins were observed in abundance in the peel extract of *Ficus* highlighting the need for future research studies to understand the fig extract's antioxidant action mechanism.

Mujic et al. (2012) examined the active ingredients in extracts from five different fig cultivars 'Araguja', 'Termenja', 'Crnica', 'Bjelica', and 'Bruetka bijela" in a research study. They determined the antioxidant capacity by applying a DPPH assay and quantify some antioxidants through the high-performance liquid chromatography (HPLC) technique. The highest phenolic content was detected in methanolic extract i-e 7.24 to 11.7 mg in dried sample extract. For the extract cultivar "Crnica," the DPPH assay results were found to exhibit an IC50 value at extract concentrations lower than 0.40 mg/ml, whereas for other cultivars, this value was higher than 0.60 mg/ml. The antioxidant test's results showed that "Bjelica" had higher antioxidant activity than all

other extracts. Results from the reducing power assay were compared to the standard antioxidant compound ascorbic acid's activity.

Since ancient times, phenolic compounds made by plants have been recognized as potential antioxidants and free radical scavengers. Researchers have shown a great deal of interest in the traditional applications of traditional medicines against conditions linked to oxidative stress. The antioxidant capacity and phytochemical content of four varieties of *Ficus carica* fruits, fig flowers, or "Bakor," as they are known locally in Algeria, were compared in a study by Mohammed et al. (2020). The antioxidative potentials of plant extracts were determined using both total antioxidant capacity and the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging test, followed by quantification of phytochemicals to determine the entire flavonoid, anthocyanin, tannins and phenolic. The amount of phenolics and the antioxidant strength of fig flower methanol extracts were found to be positively correlated. Methanolic extract of bechar (MeOH Var.1) was found to contain the highest amount of phenolics, 10.4 mg GAE/g DW.

The compositions of phenolics and reducing sugar in 27 Tunisian ecotypes of figs were investigated by Aljane et al. (2020) in their research study. Additionally, the ABTS and DPPH assays were also applied to measure the antioxidant activity. DPPH inhibition percentages ranged from 11.37 ('Besbessi') to 64.73% ('Bouharrag'), and ABTS inhibition percentages ranged from 38.50 (Sawoudi5) to 676.13 ('Nemri'). The ecotypes 'Zergui' and 'Nasri' had shown to contain the highest glucose and fructose contents (5.68 and 4.83 g/100 g FW, respectively) and fructose content (5.43 and 4.69 g/100 g FW, respectively). The findings also revealed that fig fruits are a beneficial source of naturally occurring antioxidants that could be employed in the food and pharmaceutical industries.

Çalişkan et al. (2011) investigated the phytochemical properties and antioxidant potential of green, yellow, brown, purple, and black fruit fig (*Ficus carica* L.) accessions. In this study, variables (like L*, a*, C*, and hue) describing fruit skin colors as well as total phenolics, glucose, fructose, sucrose and anthocyanins were also examined. FRAP assay was used to determine the antioxidant properties of fig fruits. The polyphenol and anthocyanin contents of fruits were found to be significantly correlated with the antioxidant ability of fig (i.e 0.74 and 0.63, respectively). These black-fruited accessions outperformed green and yellow fig accessions in terms of total antioxidant capacity, anthocyanin, and phenolic content by a factor of 2, 15, and 25. However, compared to other color groups, the fructose, glucose and sucrose content of brown and purple fig accessions was higher. According to HPLC results, fructose (56%) and glucose (43%) were the two main sugars reported.

Ercisli et al. (2012) conducted a study on fresh fruits of several local and well-known fig (*Ficus carica L.*) genera and cultivars widely cultivated in northeastern Turkiye, the color of the fruit skin, the total phenolics and anthocyanins, the content of soluble solids, the titratable acidity, and the total antioxidant capacity were determined in this research. In general, antioxidant capacity

capacities were assessed by the DPPH and ABTS methods and found that the antioxidant values were higher in local fig genotypes than in cultivars. The findings also provided information on the potential health benefits of locally grown genotypes and recommended that genetic makeup is the primary factor that influences variations in the composition of bioactive substances in figs.

Another study by Debib et al. (2014) deals with four different extracts of two varieties of dried figs (*Ficus carica* L.) Azendjar and Taamriout to find out the in vitro antioxidant and antimicrobial potential by applying DPPH radical scavenging activity assay and agar diffusion method as well as minimum inhibitory determination method. Furthermore, the extracts were tested for polyphenolics, flavonoids, and tannins using the Folin-Ciocalteu assay, the aluminum trichloride method, and casein precipitation, respectively. The methanol extract of the Azendjar variety demonstrated the highest antioxidant capacity, with an IC50 of 0.080 mg/mL. All extracts had varying degrees of antimicrobial activity against the Gram-positive and Gram-negative bacteria tested. *Candida albicans* emerged as the most sensitive to all of the extracts. The methanol extracts of the Taamriout variety demonstrated significant antimicrobial activity (MIC values 32-128 mg/mL). Furthermore, the findings revealed that phenolics and flavonoids were prevalent in acetone and aqueous extracts.

2.4. Transcriptomic of *Ficus carica L.*

The *Ficus carica L.* (Moraceae) originally belongs to the largest angiosperm genera and has a distinctive closed inflorescence structure. Fresh figs are perishable and must be handled with care after harvesting. Investigating the pathways that contribute to fig ripening may reveal new ways to extend their storage life. Analyzing fig ripening pathways may reveal additional strategies for prolonging their storage life. To identify the main active metabolic pathways and transcription factors during fig fruit ripening. Freiman et al. (2014) investigated four developmental phases of fig fruit for transcriptome sequencing and analysis. They discovered 46,927 singletons, 39% of which had a matching sequence from the nonredundant database, and 12,751 unigenes, 93% of which were structurally similar to at least one nonredundant database sequence. Variable activities related to photosynthesis, the metabolism of anthocyanins and volatile compounds, the metabolism of cell walls and wax, the expansion of cells, transcription, and the metabolism and organization of DNA was identified. Genes for ethylene synthesis were also found. Finally, 516 unigenes that encode transcription factors and were involved in the control of the early and late fruit maturation processes were discovered. The identification of three genes that encode members of the *AGL2* (SEP) subfamily, which is closely related to ripening regulation, was made possible by concentrating on eight *FcMADS*-box transcriptional regulators.

Despite being evolutionarily conserved, the plant CP genome contains highly variable regions, some of which show an accelerated rate of evolution (Zhang et al., 2017). Focusing on these features, the CP genome is frequently being used in evolutionary studies and has recently been shown to be useful for evaluating species-specific genetic markers, such as DNA barcoding and SNPs (Daniell et al., 2016).

Huang et al. (2022) determine the complete plastome sequence of ten *Ficus* species through NGS. According to comparative genomics, these plastomes have a typical quadripartite structure that is relatively conserved, and they identify potential molecular markers with eight mutation hotspots for the identification of *Ficus* species. The phylogenomic analysis clarified the taxonomy of the species, demonstrating the relationship between the *Ficus* and *Morus* genera, as well as the division of the *Ficus* genus into other subgenera (*Ficus, Sycomorus* and *Urostigma*). Overall, these findings added data to the CP genome of the genus *Ficus* as well as provided supplementary information for future *Ficus* systematics and phylogenetic reconstruction.

Wang et al. (2017) carried out metabolomic and transcriptomic combined analysis for Green Peel and its color mutant "Purple Peel". In contrast to the young "Green Peel," the young "Purple Peel" showed a significant and simultaneous upregulation in pathways of the flavonoid and anthocyanin components and relevant transcriptional factors. This upregulation was also seen in the genes encoding the enzymes involved in the phenylpropanoid and flavonoid biosynthetic pathways. The discussion covers the function of *R2R3-MYB* transcriptional regulators in the color morph mutation and possible connections to retrotransposon activity. The mature mutant also exhibited widespread upregulation of small heat-shock protein genes. Furthermore, the mature mutant showed large-scale upregulation of small heat-shock protein genes. This is the first study to show the extensive metabolome and transcriptome connectivity alteration underlying a fig mutation in a solitary horticultural characteristic, as well as the significant impact on fruit nourishment and quality. The discussion covers the function of *R2R3-MYB* transcriptional regulators in the color morph mutation and possible connections to retrotransposon activity. The mature mutant also exhibited widespread upregulation of small heat-shock protein genes. Furthermore, the mature mutant showed large-scale upregulation of small heat-shock protein genes. This was the first study to show the extensive metabolome and transcriptome connectivity alteration underlying a fig mutation in a solitary horticultural characteristic, as well as the significant impact on fruit nourishment and quality.

Cui et al. (2019) provide useful candidates for research on fruit-quality formation control and fig cultivar innovation to meet market demand by presenting the delineation of intra and inter-omic modifications in the expression of particular proteins and genes of TR vs. CR fig receptacle. From the CR to the TR stages, there is a striking increase in size, weight, flavor and sugar content along with a decrease in the firmness of the fruit. Combining quantitative metabolomic and transcriptomic data, their findings showed that ficins, sugar concentration, cell-wall alteration, the

ethylene pathway, and certain other biological procedures related to fruit quality and ripening are all differentially regulated. The multi-omic outcomes offer important insights into the fig ripening process, particularly the highlighted biomolecular basis of the final configuration of optimal usage quality, which may help with fig cultivar innovation and breeding to best meet consumer and market demands.

Metabolomics of the 'Zibao' fig fruit tissue before and after bagging was analyzed by Wang et al. (2021) using HPLC MS/MS and RNA-seq to unveil the metabolic variations of the edible portion of figs and downfield gene expression network modifications. This study provided detailed data about the composition and abundant supply of metabolites in the tissues of female fig flowers. This study provided detailed data about the composition and abundant supply of metabolites in the female flower tissue of fig. The findings revealed that modifications in the amount and availability of sugars, organic acids, peptides, and phenolics could explain differences in flavoring agents of the fruit because of bagging. This study adds to our understanding of the impacts of bagging on alteration in the intrinsic and visual quality of fruits.

Growing evidence suggests that transcription factors are critical in modulating color changes (Allan et al., 2008). It is widely known that Transcriptional elements, basic helix-loop-helix (bHLH) proteins, and WD40 proteins, specifically *R2R3-MYBs*, regulate the synthesis of anthocyanins at the transcriptional level. By activating all of the anthocyanin biosynthetic genes, the upregulation of *AtPAP1 (AtMYB75)* or *AtPAP2 (AtMYB90)* in purple recombinant tobacco plants significantly increases anthocyanin contents (Borevitz et al., 2000). *MYBs* involved in the regulation of anthocyanin biosynthesis have been found and described in several fruit species, including the apple and grape. For instance, the *R2R3-MYB* genes *MdMYB1* and *MdMYB10*, which are connected to the regulation of anthocyanin in apples, were isolated from various apple cultivars and both were found to be capable of promoting anthocyanin accumulation (Espley et al., 2007). A similar function in grapes was also reported for *VvMYBA1* and *VvMYBA2* (Jiu et al., 2021).

However, little is known about the *MYB* regulators for gene coding genes in fig. Mostly in the field of molecular biology, research on its anthocyanin biosynthesis and regulatory mechanisms is rare. Only *FcANS1* has been cloned so far, providing sequence information about the pathway of anthocyanins in figs (Cao et al., 2016). In a study conducted by Cao et al., (2016) an *FcANSI* gene was cloned from the peel of fig syconia. The results of the study revealed that the expression of *FcANSI* gene is involved in the deposition of anthocyanin. Furthermore, the expression of *FcANSI* gene was found to be responsive to natural light whereas, after bagging treatment the expression levels of the gene changed in the peels and female flowers respectively.

Likewise, a study was performed by Lama et al. (2020) on (*Ficus carica* L). The Brown Turkey variety was selected for the Abscisic acid, fluridone and ethephon treatment for the quantification of anthocyanin accumulation. The results revealed that when *FcMYB113* gene from the MBW complex is co-expressed with *FcGL3* and *FcWD40* during the late phase of anthocyanin biosynthesis, it may play a significant role in color development. The results further revealed that the ABA can start the biosynthesis of anthocyanin. Song et al. (2021) experimented on the purple-peel of fig fruit. They identified 118 basic helix–loop–helix (*bHLH*) genes. Gene expression analysis was carried out. Sixteen basic helix–loop–helix genes were selected as candidate genes associated with anthocyanin biosynthesis. The results of the study revealed the first (*bHLH*) gene involved in the fruit color development of fig. This study may enhance the future understanding of the (bHLH) gene for other significant *Ficus* species as well. Transcriptomic analysis was also performed by Li et al. (2020) on fig peels of red and yellow stages. When the red stage was compared to the yellow stage, we discovered 6224 genetic variations, specifically anthocyanin-related genes such as *5 CHS*, *3 CHI*, *3 DFR*, *3 ANS*, *2 UFGT*, and *7 R2R3-MYB* genes. Besides that, 3 anthocyanin biosynthetic genes, *FcCHS1*, *FcDFR1* and *FcCHI1*, as well as *2 R2R3-MYB* genes, *FcMYB21* and *FcMYB123* had been cloned; sequence analysis and molecular characteristics revealed their critical roles in the biosynthesis of fig anthocyanin. Further indicating their regulatory functions in fig coloration, recombinant proteins expression of *FcMYB21* and *FcMYB123* substantially increased anthocyanin concentration both in apple fruits and calli. These findings shed new light on the molecular mechanisms underlying the biosynthesis of fig anthocyanin and coloration, paving the way for the genetic transformation of high anthocyanin-containing cultivars and other agronomic traits in fig fruits.

FcMADS9, the first anthocyanin-related MADS-box gene isolated and characterized from fig peels by Li et al. (2021). The outcomes of the *FcMADS9* expression profile in fig and transient *FcMADS9* overexpression in apple peels and calli reveal that *FcMADS9* plays a positive regulatory function in anthocyanin biosynthesis. Besides, figs fruit and *FcMADS9*-overexpressed apple calli were treated with exogenous ethephon (ETH, an ethylene generating system) or 1-methyl cyclopropane (1-MCP, an ethylene inhibitor) to study the connection among *FcMADS9*-induced anthocyanin formation and ethylene. This research broadens the understanding of the molecular processes involved in fig anthocyanin accumulation, which benefits the fruit's quality.

3. MATERIALS AND METHODS

3.1. Chemicals

The HPLC standards of phenolics and sugars were acquired from Sigma-Aldrich (Steinheim-Germany). Furthermore, formic acid, acetonitrile, potassium persulphate, 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH) and 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were obtained from Merck (Gernsheim, Germany). All of the chemicals and solvents used were of analytical or HPLC quality.

3.2. Plant material and sample preparation

A total of four Turkish figs (*F.carica* L.) cultivars 'Sarilop' (greenish-yellow), 'Yesil Bozova' (greenish-light purple), 'Bursa Siyahi' (dark-purple), 'Izmir Bardakci' (yellowish-green), and four Turkish selected genotypes '01-1N-58' (dark-purple), '31-N-05' (dark-purple), '01-1M-02' (greenish-purple), and '01-1N-51'(dark-purple) were screened for the total phenolic compositions, sugars and total antioxidant capacity (Figure 3.1).

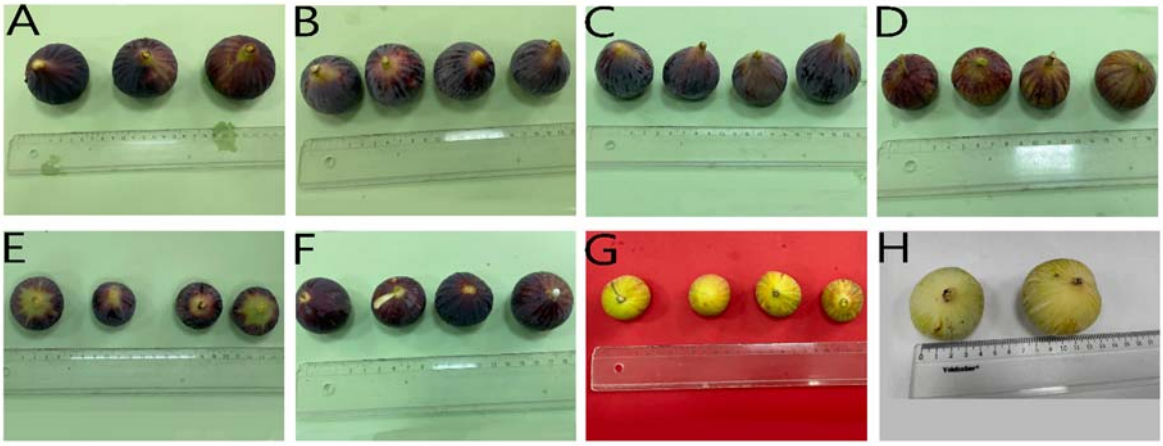


Figure. 3.1. Cultivars and genotypes of fig used in the study: (A) Bursa Siyahi (B) 01-1N-58 (C) 01-1N-51 (D) Yesil Bozova (E) 01-1M-02 (F) 31-N-05 (G) Izmir Bardakci (H) Sarilop

Fruits were collected at the tree ripening stage in an icebox directly from the Fig Genetic Collection Orchard of Çukurova University (37°01'46.6''N-35°21'46.6''E), Adana, Turkiye during August and September 2021. At least 15-20 healthy fruits per cultivar and genotype were selected based on various colors and their economic importance (Figure 3.2). Each cultivar and genotype comprised three replications. Soon after, the samples were transported immediately to the Molecular Breeding Laboratory of Çukurova University, Faculty of Agriculture Adana, Turkiye within 20 minutes to peel off the selected fig genotypes. The fruits were cleaned with 70 % ethanol and then peeled with the help of a scalpel, and the peel was detached from the pulp separately (Figure 3.3). The skin and pulp samples were immediately wrapped in aluminum foil

and dipped in liquid nitrogen, and immediately kept at -20°C and an additional quantity of samples were kept at -80 °C for later experimental use. Freezing drying of the eight (peel and pulp) samples of fig genotypes were performed in a lab-scale freeze dryer (Free Zone Benchtop Freeze Dryer, Model: 71020 2.5 L-84C series, Labconco Corp., Kansas City, MO, USA) (Figure 3.4).

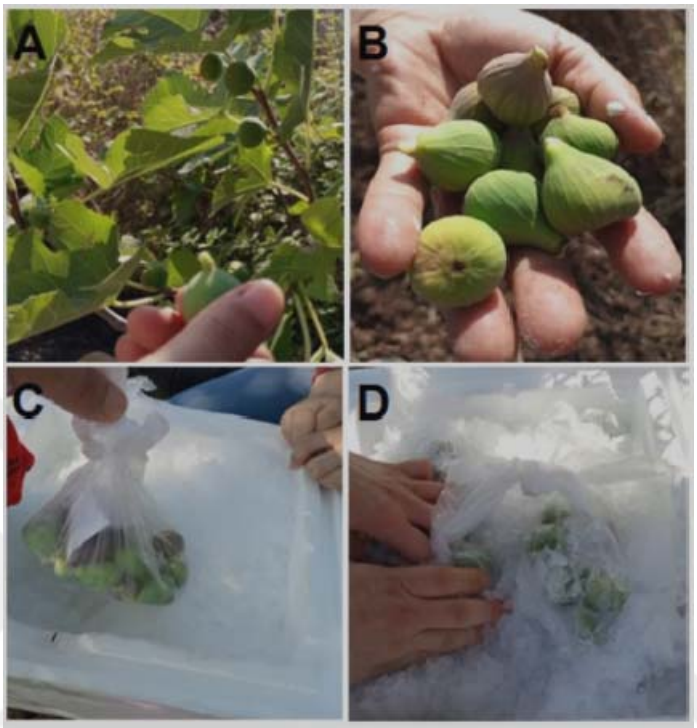


Figure. 3.2. Collection of fig samples from the field
(A) and (B) Detaching of fig fruits from trees (C) and (D) Collection of fig fruit samples in an icebox.



Figure. 3.3. Peeling of fig samples
(A) and (B) Peeling of green and yellow fig fruits skin (C) Peeling of dark purple fig fruit peels.

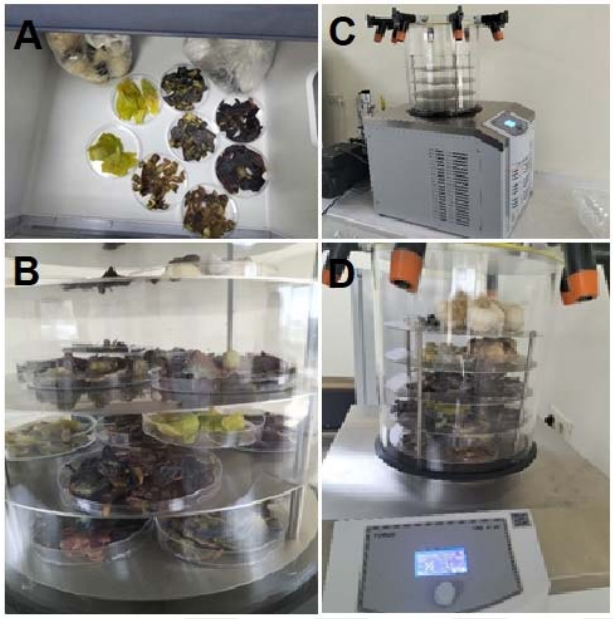


Figure. 3.4. Freezing drying of fig samples in the freeze dryer
(A) and (B) Freezing drying of fig fruit peels in freeze dryer machine (C) and (D) Freezing drying of fig whole fruits in the freeze dryer machine

3.3. Phenolic compounds extraction and HPLC-ESI-MS/MS analysis

The phenolic compounds (PCs) extraction was performed according to Vichi et al. (2007) with some modifications. The freeze-dried samples were crushed with an IKA mixer blender machine (Figure 3.5). Ten grams of powdered samples were taken (Figure 3.5). 80% methyl alcohol (MeOH) was prepared as a solvent and added to the samples (Figure 3.6). The extracted supernatant was injected into the vial bottle up to 1.5 mL with the help of a syringe. Before injection, a 0.45 µm pore size filtrate was placed above the vial bottle (Figure. 3.9). Two parallel samples were prepared for each genotype for the High-Performance Liquid Chromatography (HPLC) analysis of PCs (Figure 3.9). For the analysis of the PCs, an Agilent 1100 HPLC system (Agilent Tech, Palo Alto, CA-USA) accompanied by a diode array detector was used with ChemStation software operated by Windows NT. For separation, a Beckman Ultrasphere ODS column (Roissy, France; 4.6 mm 250 mm, 5 m) was used (Figure 3.10). The mobile phase was comprised of water having 5% formic acid as (solvent A; v/v) and acetonitrile with 40% solvent A (solvent B; v/v) respectively. The elution procedure was carried out exactly as reported by Kelebek et al. (2020). An Agilent 6430 LC-MS/MS spectrometer armed with an electrospray ionization (ESI) source was utilized to validate, identify, and assign each compound by comparing retention time and UV spectrum to legitimate standards. In the negative ion mode, the subsequent parameters were used: drying gas at 12 L/min, capillary temperature at 400 °C, and 45 psi nebulizer pressure of ESI/MS. Acquisition of data was accomplished using the MRM (multiple reactions monitoring), which recognizes certain mass transitions in the MS-MS–negative ion mode at predetermined retention times. Standard calibration curves of phenolics were applied for quantification purposes as described by Kelebek et al. (2015). The quantification and screening limits were measured employing the S/N values of 10 and 3, respectively.



Figure. 3.5. Extraction of Phenolic compounds
(A) Crushing of fig samples in IKA blender machine (B)-(F) Collection of powdered fig samples in falcon tubes

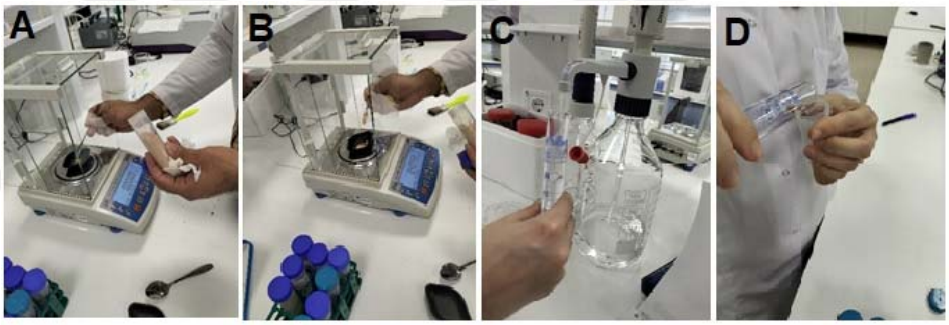


Figure. 3.6. Weighing and preparing solvent for the extraction of Phenolic compounds
(A-B) Weighing two grams of powdered samples (C-D) Preparing methyl alcohol solvent



Figure. 3.7. Different colors of fig peels and pulps
(A-D) Exhibits the various colors of fig genotypes obtained after adding solvents

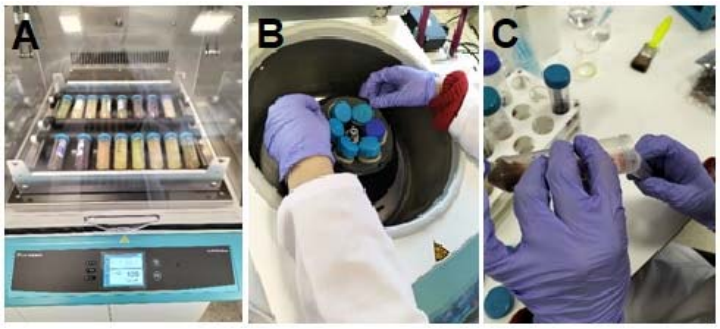


Figure. 3.8. Homogenization of fig samples
(A) Fig samples were placed in shaker (B-C) Centrifugation of fig samples

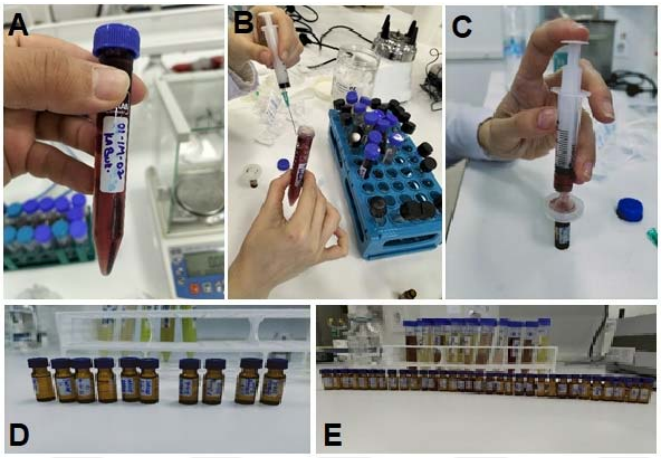


Figure. 3.9. Preparing the fig samples for HPLC-MS-MS analysis
(A) Supernatant transferred into small falcon tubes (B) The supernatant transferred via syringe into vial bottle (C) Transfer of supernatant via filtrate into vial bottle (D-E) The vial bottle samples ready for the HPLC analysis



Figure. 3.10. An Agilent 1100 HPLC system for the analysis of the phenolic compounds

3.4. Sugars extraction and HPLC analysis

Sugar extractions were carried out using the method of Jiao et al. (2010). Prior to injection, a 0.45 m pore-size membrane filter was used to filter the sample. To analyze sugars, an HPLC system (LC-10A HPLC Series; Shimadzu, Kyoto, Japan) was used that contains a pump system and a refractive index detector (RID-10A; Shimadzu) (Figure 3.10). A column of Aminex HPX-87H (300 × 7.8 mm) (Bio-Rad, CA, USA) was used to analyze sugars simultaneously at 55°C. The analytical conditions were optimized as follows: flow rate was 0.4 mL/min and eluent concentration was 0.09 mol/L of H_2SO_4 with 6% acetonitrile (v/v). The chromatographic peak associated with each sugar was detected by comparing the retention time to a standard. A calibration curve has been primed using standards for determining the association between peak area and concentration.

3.5. Antioxidant assays

3.5.1. DPPH analyses

The 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH) test was carried out as reported by (Kelebek et al., 2013). Each dilution of the extract (1, 20 and 50 mg/mL) in ethanol/water (v/v) was prepared three times. A 0.1 mL aliquot of the diluted extracts was added to 3.9 mL of DPPH solution in methanol (6 × 10−5 M) (Figure 3.13). The mixture was briskly mixed and allowed to stand for 30 minutes at room temperature. Using a Cary 60 UV/VIS spectrophotometer (Agilent Technologies, Santa Clara, California, USA), the solution's absorbance was measured at 517 nm (Figure 3.11).

3.5.2. ABTS analyses

The 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay was carried out according to the method described by Brand-Williams et al. (1995) with some modifications. A radical cation of ABTS was prepared by reacting 7 mM ABTS with 2.54 mM potassium persulfate, following 12–16 hours of incubation at room temperature. The ABTS solution was diluted with ethanol before the assay, resulting in absorbance at 734 nm. Each 0.1 mL sample was diluted with 3.9 mL of ABTS solution (Figure 3.12). The reaction mixture was left for 30 minutes at room temperature, and the absorbance at 734 nm was measured (Figure 3.11).

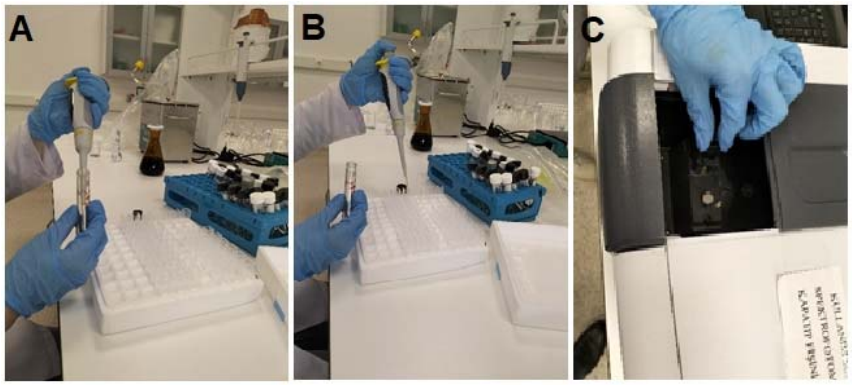


Figure. 3.11. Preparing the dilutions for antioxidant analysis
(A-B) Transfer of dilutions with the help of micro-pipette in cuvette (C) Spectrophotometer analysis of the dilutions

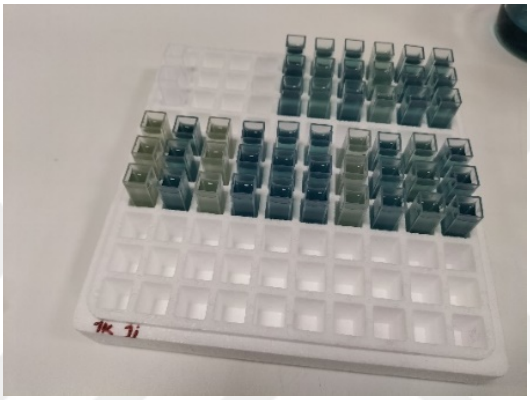


Figure. 3.12. ABTS antioxidant analysis of fig samples depict blue color in cuvettes

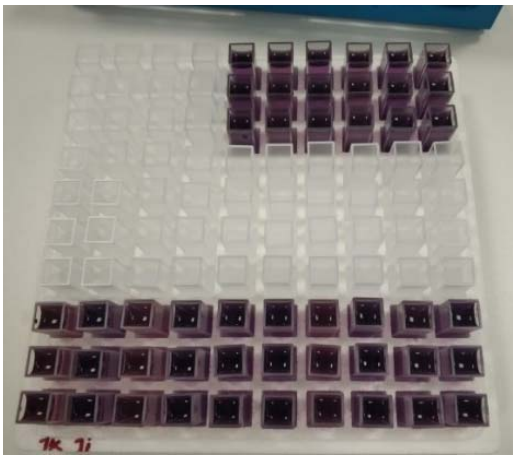


Figure. 3.13. DPPH antioxidant analysis of fig samples depict deep purple color in cuvettes

3.6. RNA isolation

The peels of two fig varieties '01-1N-58' exhibits the maximum anthocyanin content and 'Sarilop' represents the lowest anthocyanin content stored at -80ºC which were further used for RNA isolation. The peels were ground with the help of liquid nitrogen in a mortar and pestle and transferred into Eppendorf tubes. In addition, two different extractions for isolation buffer have been prepared. Extraction Buffer I: 2% CTAB for preparation of this solution (Cetyltrimethylammonium bromide), 25 mM EDTA (Ethylenediamine tetraacetic acid), 100 mM Tris-HCL (Tris (hydroxymethyl) aminomethane hydrochloride; pH: 8), 2.0 M NaCl (Sodium chloride), 0.5 g/L Spermidine and 2% PVP (Polyvinylprolidon; MW: 2.5g/mol) was used. The solution was autoclaved at 121°C for 20 minutes. The 50 µg/ml proteinase K was added before use. Extraction Buffer II: 2% CTAB for preparation of this solution (Cetyltrimethylammonium bromide), 1 mM, 10 mM Tris-HCL (Tris-hydroxymethyl) aminomethane hydrochloride; pH:8), 1.0 M NaCl (Sodium chloride), 0.5 g/L Spermidine and 0.5% SDS (Sodium dodecyl sulfate) was used. Furthermore, the Extraction Buffer II was Autoclaved at 121°C for 20 minutes and up to 65°C before use has been heated. Other Solutions were also prepared: Chloroform isoamyl alcohol for RNA isolation (24:1), 10 M LiCl (Lithium Chloride), 70% EtOH (prepared with DEPC pure ethanol), C3H8O (Isopropyl alcohol prepared with DEPC), 3 M NaOAc (Sodium Acetate, pH: 5.2), TE buffer (Tris-EDTA) solutions were prepared and stored at 4°C. The RNA isolation was done according to (Zarei, 2012) with some modifications.

3.6.1. RNA isolation steps

- The peels of the two fig varieties '01-1N-58' and 'Sarilop' were ground with the help of liquid nitrogen in a mortar and pestle and transferred into Eppendorf tubes containing 150 mg/L PVP (Polyvinylprolidon).
- The 1 ml of Buffer I heated at 65°C was added to each tube. The tubes were incubated at 65°C for 40 minutes with rapid mixing. The tubes were mixed rapidly every 5 minutes and homogenized (Figure 3.14).
- Chloroform; Isoamyl alcohol (24:1) was added to the tubes and the tubes were thoroughly mixed (Figure 3.16).
- The tubes were centrifuged at 20,000 rpm for 30 minutes. The supernatant was transferred to 1.5 ml Eppendorf tubes (Figure 3.15).
- A 3:1 ratio of LiCl (Lithium Chloride) was added to the tubes at 4°C and was incubated at least for 16 hours.
- The samples taken from the incubation were centrifuged at 20,000 rpm for 30 minutes.
- The supernatant is poured carefully and 1 ml of 96% EtOH (Ethanol) is added to the pellet was added.

- The samples were centrifuged at 20,000 rpm for 5 minutes.
- Alcohol was carefully removed from the samples and pellets were left to dry at room temperature.
- The 750 µl, pre-warmed 65°C extraction buffer II was added to the pellet.
- Samples were incubated at 65 °C for 30 min.
- A total of 750 µl of 24:1 ratio of Chloroform Isoamyl alcohol in the tubes were added.
- The samples were centrifuged at 20,000 rpm for 30 minutes and the supernatant was taken and freshly transferred to the tubes.
- 100 µl of NaOAc (Sodium acetate) and 600 µl of C3H8O (Isopropyl alcohol) were added to the supernatant and the samples were mixed gently.
- Samples were kept at -20°C for at least 2 hours.
- The samples were centrifuged at 20,000 rpm for 30 minutes.
- The liquid part of the samples was removed to allow the pellet to settle down at the bottom.
- 1 ml of 70% ethanol and 30 µl of NaOAc (Sodium acetate) were added to the pellet.
- The samples were centrifuged at 15,000 rpm for 5 minutes and the liquid part was discarded and the pellet remains at the bottom of the tubes.
- 1 ml of 70% EtOH (Ethanol) was added again.
- The samples were centrifuged at 15,000 rpm for 5 minutes.
- Alcohol from samples were removed and the pellet was left to dry (Figure 3.17).
- A total of 30 µl of TE Buffer (Tris-EDTA) was added to the pellet.
- The RNA obtained from two samples were stored at -80°C.

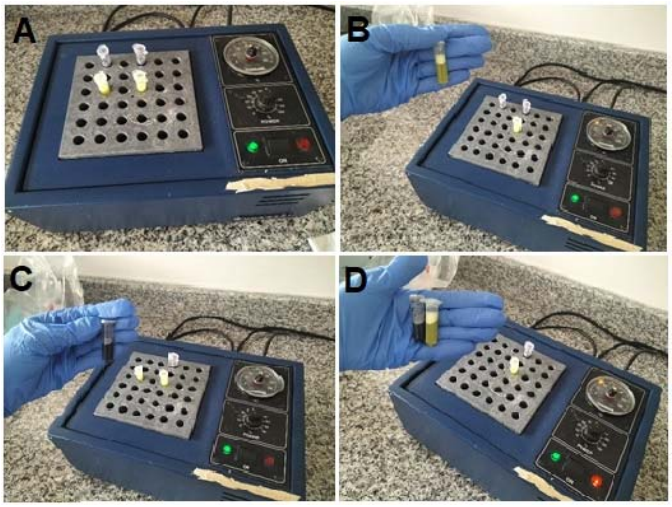


Figure. 3.14. Incubation of fig samples in water bath
(A and D) Sarilop and 01-1N-58 fig genotypes incubated in water bath (B) Sarilop depicts green color incubated in water bath (C) 01-1N-58 depicts dark purple color incubated in water bath

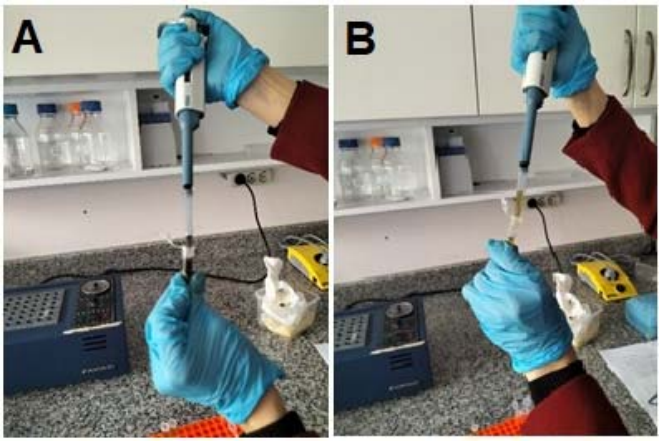


Figure. 3.15. Transfer of supernatant from the two fig genotypes
(A) Transfer of supernatant from 01-1N-58 fig genotype (B) Transfer of supernatant from Sarilop fig genotype

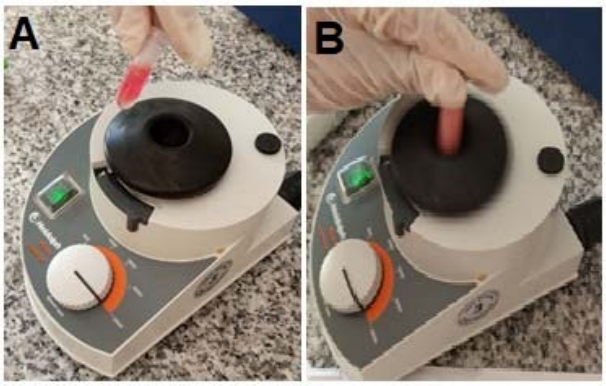


Figure. 3.16. Vortex the fig samples for homogenization
(A and B) Fig sample is ready for vortexing

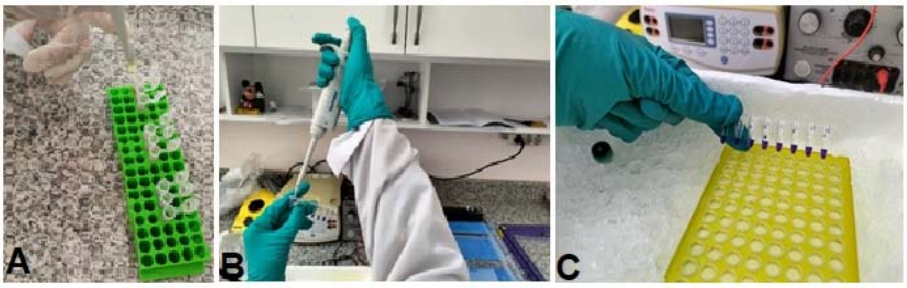


Figure. 3.17. Various steps involved during RNA isolation
(A) Air drying of fig samples during RNA isolation (B) Samples preparation for gel electrophoresis (C) RNA mixed with the dye for loading in gel electrophoresis

3.6.2. Determination of quality and quantity of RNA samples

The purity and concentration of RNA was determined by a Nanodrop spectrophotometer. Furthermore, the Aglient 2100 Bio Analyzer (Agilent RNA 6000 Nano Kit) device was used to measure the RIN value of the isolated samples, to determine the degree of purity and to determine the length of the fragments during the fragmentation phase. Additionally, the RNA integrity was confirmed by the sharpness of ribosomal RNA bands visualized by electrophoresis which showed distinct 28S and 18S bands, indicating that the RNA was not degraded and was also relatively free

of RNases (Figure 3.17). For this, 1.5 % of agarose gel was prepared. RNA was isolated and 5 µl of the samples were taken and 1µl of 6X loading dye and 5 µL of ddH2O were added onto it and run at 120 volts for 40 minutes. At the end of the time, the agarose gel was viewed under UV light.

3.6.3. RNA sequence (RNA-Seq) analyzes

mRNA isolation and mRNA fragmentation

RNA sequencing studies were carried out at Genoks Genetic Diagnosis Center, Ankara, Turkiye. Total RNA was isolated from the tissues used in the study. The mRNA isolation was performed using total RNA samples isolated before sequencing. The Oligo dT technique was used to isolate mRNA from total RNA samples. In order for the isolated mRNAs to have specific fragment ranges, they were subjected to fragmentation. The mRNA fragments obtained by the fragmentation process were used to construct cDNA libraries.

3.6.4. Preparation of cDNA libraries

Using mRNA fragments as template, cDNA synthesis was carried out. EB (10 mM Tris-Cl, pH 8.5) buffer was used to repair the synthesized short cDNA chains and add adenine nucleotides. Short cDNA chains were purified by dissolving in EB buffer. After processing, adapters were attached to the cDNA fragments. Thus, cDNA libraries to be used in sequencing were created. cDNA libraries were sequenced using the Illumina Nova-Seq™ 6000 sequencing platform (Figure 3.18).

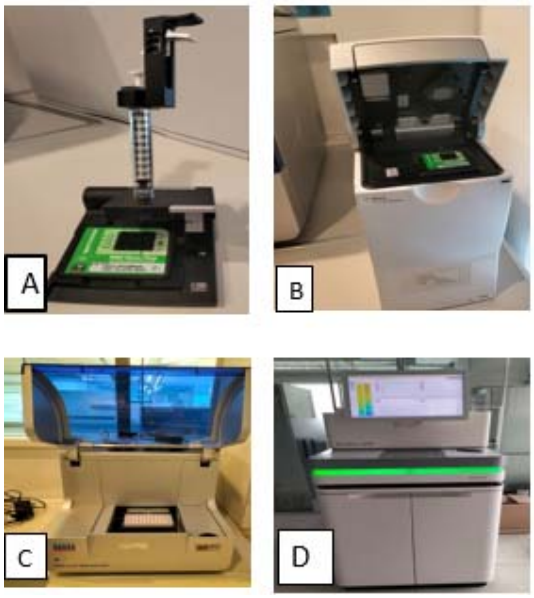


Figure. 3.18. Various steps involved during RNA sequencing
(A) RNA Nano Chip with injection (B) Agilent 2100 Bioanalyzer (C) QIAxcel Advanced electrophoresis system (D) Illumina Nova-Seq™ 6000 sequencing platform

3.6.5. Filtering sequences

Sequence reads obtained after sequencing are referred to as raw data. Reads were filtered to remove adapter sequences, poor quality reads and unread regions from the raw data obtained after sequencing. By using SOAPnuke (SOAPnuke version vl.5.2 software) program for filtering,

1. Removal of adapters from sequences,
2. Removal of unread or incorrect readings from the sequences,
3. Removal of low-quality reads from the sequences (reads with less than 10% quality) was performed.

After filtering, the raw sequences are expressed as clean sequences or reads (Clean Reads). Bioinformatic analyzes were performed using the clean reads obtained.

3.6.6. Bioinformatics analysis

Mapping sequences to the reference genome

Clean reads obtained by sequencing were mapped to the figs (*Ficus carica* L.) genome. Access to genome information via NCBI database provided. H1SAT for mapping (H1SAT version: v2. 0.4 software) program has been used. After the genome mapping process, the StringTie (Sitring Tie vl: 0.4 software) program was used for the analysis of the Resequence, and the Cuffcompare (Cufflinks version: v2.2.1 software) program was used for the determination of the obtained transcripts and their annotation to the reference genome. With the Cuffcompare program, the identification of new transcripts, the identification of unknown intergenic transcripts, the identification of transcripts that seem to be included in the reference genome intron region, the identification of exonic regions overlapping with the reference transcript, and the detection of new isoform transcripts are provided. Downstream analysis was performed with the CPC (CPC version: v0.9-r2 software) program. The GAK program was used to determine the SNP and INDEL regions in the analyses. The rMATS (rMARS version: v3.2.5 software) program was used to determine isoform transcripts, to determine the density of isoform transcripts and to determine the expression level of these transcripts.

Gene expression analysis

RSEM (version: v 1.2.12) package program was used to determine the gene expression level of clean reads mapped to the reference genome. With the program, the expression levels of the genes available in the RNA-Seq data and the expression levels of isoform genes were calculated. With the package program used, it was ensured that the correlation between the samples was determined, hierarchical clustering analysis was performed and the diagrams of the samples were created.

Identification of genes expressed at different levels (DEG)

Changes in gene expression levels between libraries were analyzed according to the FPKM (Fragments Per Kilobase of Millions Mapped) value.

FPKM value:

Calculated using the formula FPKM = (106XC)/ (NL/103).

C: The number of fragments that match the reference genome,

N: The number of fragments that match all genes,

L: base number of target gene,

The FPKM value allows the calculation of the expression level of all genes present in the cDNA libraries and the differences in the expression level that occur between genes. The DEseq2 program analyzes based on the FPKM value. DEseq2 program was used to determine the genes expressed at different levels among the samples and to analyze the expression level of these genes. With this program, the expression level of genes belonging to all samples, differences in expression levels between samples, and differences in isoform gene expression levels were calculated. In addition, cluster analyzes were performed based on the gene expression level of the genes that were expressed at different levels among the samples.

Gene ontology (GO) analysis

Gene ontology analyzes were performed to obtain information about the biological process, cellular component, and molecular function, in which genes expressed at different levels among the libraries are involved. Phyper, a function of the R package program, was used for gene ontology analysis. As a result of the analysis, genes with different expression levels were grouped according to their functions.

Metabolic pathway analysis of genes (KEGG analysis)

KEGG (Kyoto Encyclopedia of Genes and Genomes) analyzes were performed to determine which metabolic pathways are associated with genes expressed at different levels among the samples. Phyper, a function of the R package program, was used for KEGG analysis.

3.6.7. Quantitative real time PCR (qRT PCR) analysis

As a result of RNA-Seq analysis, 4 genes and 1 endogen control were selected. Selected genes were used in qRT-PCR reactions for validation of RNA-Seq data. Primers of selected genes were designed for use in qRT-PCR reactions.

Designing primers used in qRT PCR analyzes

Specific primers for the gene regions of the determined genes were designed in order to use the important genes as a result of RNA-Seq in qRT-PCR reactions. Primers were designed using the Primer3 program (Rozen and Skaletsky, 2000).

qRT-PCR (real time PCR) analysis

Associated with anthocyanin biosynthesis in quantitative real-time PCR studies 4 different genes *PAL*, *CHS, CHI* and *F3'H* reported to be present were used. PgRPSII as endogenous control gene was used. Primers of related genes are given in Table 3.1.

Table 3.1. The various genes used in the the study according to Ben-Simhon et al, (2015)

Gene Name	Forward Primer	Reverse Primers
PAL	5'-TGGAGTGTCTCAGCAGTTGG-3′	5'-CGGAACAGCATAGGATGGAT-3′
CHS	5'CCCACTAAAGCGACCCATT-3′	5'-AGACCACAAAATGCCTCCAC-3′
CHI	5'-ACTACCATTGACGGGTGCTC-3′	5'-GTGTAAGTGCCCACGGATTT-3′
F3'H	5'-CGAGTTGATACCGTTTGGG-3′	5'-GTTCAGCTTCTCGGGCAT-3′
PgRPSII	5'TCAATTTGTGAGGGTCGTTCT-3′	5'-GATTCAAGAGTAGTAACCGATTCCA-3′

cDNA synthesis stage

The cDNA synthesis reaction was established using RNA samples from the tissues. Applied Biosystems High Capacity cDNA Reverse Transcription Kit (LOT:00756490) was used for cDNA synthesis. The reagents used for the cDNA reaction were transferred to 0.2 eppendorf tubes. The tubes with the reagents were taken to the Thermalcycler device. The PCR cycle conditions used for cDNA synthesis are 10 minutes at 25°C, 120 minutes at 37°C, 5 minutes at 80°C, and oo at +4°C. The synthesized cDNAs were stored at -20°C until the step of qRT-PCR reactions. The reagents used in cDNA synthesis and their volumes are given in Table 3.2.

Table 3.2. Reagents Used in cDNA Synthesis

REAGENT	1X (µl)
10x RT Buffers	2
dNTP Mix	0.8
Random Primer	2
Reverse Transcriptase	1
Total RNA	10
H_20	4.2
Total	20

qRT-PCR reactions

Primers of the predetermined genes and 18S rRNA gene were used in qRT-PCR reactions. In addition, SYBR GREEN (SensiFAST SYBR Lo-ROX Kit) kit with fluorescent radiation was used in the reactions. The reactions were carried out according to the kit protocol, and the PCR cycle conditions were also programmed according to the kit protocol. Cycle program used in the reactions 10 min at 95°C, 15 sec at 95°C, l min at 55°C, 15 sec at 95°C, l min at 60°C, 15 sec at 95°C . The CT values obtained as a result of the reactions were evaluated according to the $2^{-\Delta\Delta C}{}_{T}$ formula described by Livak and Schmittgen (2001). and the differences in gene expression levels between the samples were determined. The reagents used in the study and their volumetric ratios are given in Table 3.3.

Table 3.3. Reagents Used in qRT-PCR Reactions

REAGENT	1X (µl)
SYBR Green	10
Forward Primer	0.2
Reverse Primer	0.2
Template	2
H_20	7.6
Total	20

3.7. Statistical analysis

The data for total phenolic compounds, sugars and antioxidant capacities in peel and whole fruit extracts of each variety were performed by using analysis of variance (ANOVA). The experimental data presented were shown as means ± standard deviations. Significant differences were evaluated using the LSD test ($p < 0.05$). The statistical analysis was conducted using the SAS Institute, Inc.'s JMP (version 8.0) statistics software package. Furthermore, the principal component analysis (PCA) was conducted using XL Stat program trial version 2020.

4. RESULTS AND DISCUSSION

4.1. Phenolic content in the peel and pulp of fig (*Ficus carica* L.) cultivars and genotypes

Phenolics are produced through multiple pathways, including mevalonic, malonic, and shikimic acids, accompanied by methylerythritol phosphate. They consist of groups of compounds with varying chemical structures (flavonoids, flavanols, aromatic amino acids, tannins and phenolic acids) that have different activities to perform in plant tissues. Additionally, they offer mechanical strength, protection and resistance from multiple biotic and abiotic stressors as well. Based on chemical structure, quality and fruit location, they also contribute to color, flavor, and aroma, particularly in the edible parts of the fruit (Karantzi et al., 2021).

The data analysis of the total phenolic content from the peels and pulps of eight Turkish fig cultivars and selected genotypes were carried out by the HPLC-DAD-ESI-MS/MS method. In total, 31 phenolic compounds including 7 anthocyanins (Cyanidin-3,5-O-diglucoside, (Epi)catechin-(4-8)-cyanidin-3-rutinoside, (Epi)catechin-(4-8)-cyanidin-3-rutinoside, Cyanidin-3-glucoside, Cyanidin-3-O-rutinoside, Pelargonidin 3-0-rutinoside and Cyanidin-3-malonylglucoside) and 24 non-colored phenolics were detected (Figure 4.1) and the analysis results are shown in (Table 4.1) and (Table 4.2).

A similar study was conducted by Hssaini et al. (2021) on the peels and pulps of the figs where they performed HPLC-MS/MS analysis and detected 12 phenolic compounds (PCs), including both the colored and non-colored PCs. Furthermore, a similar study was conducted by Yemiş et al. (2012) on three Turkish fig cultivars and detected four anthocyanins. Likewise, a study was performed by Palmeira et al. (2019) on the Portuguese fig variety “Pingo de Mel” cultivar and detected 18 PCs from the peels and pulps.

Table 4.1. Determination of total phenolic compounds in peels of eight figs (*F.carica* L.) cultivars and genotypes

	No	Identified Compounds	RT	01-1N-58	SARILOP	Y-BOZOVA	31-N-05	BURSA SIYAHI	IZMIR BARDAKCI	01-1M-02	01-1N-51
Colored PCs-Anthocyanins	1	Cyanidin 3, 5-diglucoside	16.467	1.40±0.01^{B}	ND	0.46±0.00^{F}	0.71±0.00^{D}	1.71±0.00^{A}	0.24±0.00^{G}	1.06±0.00^{C}	0.65±0.01^{E}
	2	(Epi)catechin-(4-8)- Cyanidin 3-rutinoside	18.173	0.21±0.01^{A}	ND	0.08±0.00^{E}	0.14±0.01^{B}	0.11±0.00^{C}	ND	0.10±0.00^{D}	0.08±0.00^{E}
	3	(Epi)catechin-(4-8)- Cyanidin 3-rutinoside	20.072	0.51±0.00^{B}	ND	0.16±0.00^{E}	0.67±0.00^{A}	0.39±0.00^{C}	ND	0.51±0.00^{B}	0.21±0.00^{D}
	4	Cyanidin 3-glucoside	25.411	10.66±0.01^{A}	0.03±0.00^{H}	0.72±0.00^{F}	7.33±0.00^{B}	7.07±0.00^{C}	0.16±0.00^{G}	2.07±0.00^{E}	2.59±0.00^{D}
	5	Cyanidin 3-rutinoside	27.486	135.68±0.05^{A}	0.20±0.00^{H}	9.79±0.00^{F}	120.03±0.07^{B}	106.06±0.05^{C}	2.13±0.00^{G}	34.79±0.04^{E}	47.00±0.05^{D}
	6	Pelargonidin 3-rutinoside	32.116	2.35±0.01^{A}	ND	0.06±0.00^{F}	0.35±0.00^{E}	0.68±0.01^{D}	ND	1.08±0.01^{C}	1.14±0.01^{B}
	7	Cyanidin 3-malonylglucoside	38.635	0.46±0.01^{A}	ND	0.14±0.00^{D}	0.45±0.00^{B}	0.38±0.00^{C}	ND	0.11±0.00^{F}	0.13±0.00^{E}
	Colored PCs Mean Total			**151.25±0.1**	**0.23**	**11.40**	**129.68±0.08**	**116.40±0.06**	**2.52**	**39.73±0.05**	**51.78±0.07**
Identified Colorless PCs mg GAE/100 g DW	1	Gallic acid di-pentoside	27.16	7.62±0.01^{AB}	1.85±0.01^{B}	3.35±0.13^{B}	13.96±0.19^{AB}	15.95±22.51^{AB}	6.33±0.06^{AB}	21.78±0.19^{A}	ND
	2	Gallic acid	14.49	2.98±0.23^{D}	0.80±0.00^{F}	1.41±0.04^{E}	1.60±0.12^{E}	0.43±0.00^{G}	4.75±0.06^{A}	3.61±0.22^{B}	3.29±0.01^{C}
	3	Catechin	31.23	49.84±5.26^{B}	6.29±0.40^{E}	4.14±0.80^{E}	105.72±2.65^{A}	39.14±0.67^{C}	17.85±0.62^{D}	50.80±0.20^{B}	50.25±0.10^{B}
	4	Epicatechin	39.21	151.46±0.18^{A}	ND	15.15±0.38^{D}	119.19±4.02^{B}	97.24±1.94^{C}	12.06±0.40^{D}	102.00±11.04^{C}	126.55±0.25^{B}
	5	Prenylhydroxygenistein	22.43	6.60±0.35^{F}	20.43±0.35^{DE}	90.97±0.26^{A}	24.84±1.54^{CD}	28.24±2.18^{C}	35.70±4.60^{B}	26.84±0.83^{C}	16.70±0.03^{E}
	6	Neochlorogenic acid	25.85	5.91±0.18^{C}	0.03±0.00^{G}	13.95±0.02^{A}	4.16±0.39^{E}	12.11±0.09^{B}	5.01±0.23^{D}	2.47±0.06^{F}	4.18±0.01^{E}
	7	Chlorogenic acid	31.64	524.74±1.12^{A}	498.18±0.07^{B}	451.01±0.66^{C}	426.26±1.03^{D}	423.14±6.99^{D}	444.93±2.30^{C}	357.77±0.78^{E}	91.13±0.18^{F}
	8	Cryptochlorogenic acid	37.24	0.55±0.02^{G}	47.68±1.36^{B}	66.90±0.88^{A}	20.76±0.75^{D}	4.38±0.28^{F}	41.73±1.19^{C}	6.89±0.03^{E}	3.69±0.05^{F}
	9	Dihydroxybenzoic acid pentoside	24.92	1.83±0.01^{ABC}	1.10±0.01^{BCD}	0.54±0.03^{D}	0.79±0.00^{CD}	2.42±1.51^{A}	0.81±0.07^{CD}	2.13±0.12^{AB}	2.00±0.03^{ABC}
	10	Luteolin	64.57	2.36±0.04^{D}	0.57±0.00^{F}	1.97±0.02^{E}	3.65±0.02^{B}	4.98±0.16^{A}	0.68±0.00^{F}	2.95±0.11^{C}	2.77±0.16^{C}
	11	Dihydroxybenzoic acid di-pentoside	29.54	21.28±0.09^{A}	12.60±0.35^{BC}	13.13±0.27^{ABC}	19.59±1.32^{AB}	12.26±10.22^{BC}	5.11±0.22^{C}	13.50±0.10^{ABC}	17.67±0.39^{AB}
	12	Apigenin C-hexoside C-pentoside	43.31	29.78±0.46^{B}	23.07±1.24^{C}	20.55±2.75^{C}	12.04±0.38^{E}	7.46±0.14^{F}	47.03±1.39^{A}	15.64±0.23^{D}	22.56±0.22^{C}
	13	Quercetin glucoside-O-rutinoside	41.69	2.82±0.03^{BCD}	4.02±0.24^{A}	3.65±0.81^{AB}	3.32±0.03^{ABC}	1.03±0.32^{E}	3.30±0.50^{ABC}	2.24±0.68^{D}	2.57±0.05^{CD}
	14	Quercetin 3-O-rutinosid (rutin)	47.63	104.46±1.82^{A}	90.08±1.00^{BC}	77.82±1.19^{D}	88.48±0.11^{BC}	64.61±1.25^{E}	81.69±2.99^{CD}	77.65±10.50^{D}	90.99±0.09^{B}
	15	Hyperoside	48.01	5.93±0.05^{G}	32.66±0.09^{C}	44.12±2.00^{A}	37.81±1.67^{B}	31.38±1.70^{C}	20.29±1.27^{D}	15.16±0.94^{E}	10.79±0.34^{F}
	16	Quercetin 3-O-glucoside (isoquercetin)	49.28	14.02±0.99^{C}	5.86±0.07^{F}	37.77±0.53^{A}	10.57±0.07^{D}	36.52±6.22^{A}	26.28±1.05^{B}	7.99±3.88^{DE}	8.64±0.51^{DE}
	17	Kaempferol-3-O-rutinoside (Nicotiflorin)	43.83	4.26±0.59^{C}	6.18±1.15^{BC}	16.04±0.65^{A}	5.06±3.33^{BC}	18.67±1.27^{A}	17.72±0.84^{A}	7.53±0.66^{B}	6.08±0.26^{BC}
	18	Apigenin-6,8-C-diglucoside (Vicenin)	50.54	3.28±0.01^{B}	0.87±0.01^{B}	17.36±11.95^{A}	3.27±0.01^{B}	6.06±0.11^{B}	5.71±0.40^{B}	4.60±0.13^{B}	4.43±0.69^{B}
	19	Kaempferol 7-O-glucoside	44.29	3.34±0.07^{E}	6.27±0.02^{D}	13.03±0.90^{A}	3.13±0.03^{E}	11.79±0.03^{B}	9.17±0.02^{C}	3.48±0.08^{E}	3.59±0.26^{E}
	20	Isorhamnetin-3-O-rutinoside	45.02	6.79±0.02^{AB}	4.49±0.14^{D}	3.53±0.03^{F}	4.91±0.01^{C}	3.25±0.05^{F}	4.00±0.02^{E}	6.61±0.02^{B}	6.92±0.31^{A}
	21	Luteolin C-hexoside C-pentoside 1	40.55	1.70±0.01^{F}	1.20±0.01^{G}	6.33±0.20^{C}	2.89±0.01^{E}	10.37±0.15^{A}	6.62±0.02^{B}	3.56±0.02^{D}	2.74±0.16^{E}
	22	Luteolin C-hexoside C-pentoside 2	46.95	4.47±0.00^{G}	13.26±0.02^{B}	6.62±0.04^{C}	2.48±0.08^{H}	36.55±0.44^{A}	6.15±0.01^{D}	5.65±0.03^{E}	5.11±0.07^{F}
	23	Apigenin 8-C-glucoside (Vitexin)	35.10	22.95±24.02^{A}	11.06±0.04^{A}	15.57±0.10^{A}	15.48±6.40^{A}	18.32±0.50^{A}	11.44±0.02^{A}	3.85±0.02^{A}	13.42±0.03^{A}
	24	Apigenin 6-C-glucoside (Isovitexin)	47.48	84.94±0.84^{A}	67.38±1.17^{B}	12.86±0.11^{E}	21.93±0.10^{D}	31.65±1.56^{C}	22.22±0.18^{D}	85.50±0.29^{A}	86.10±1.25^{A}
	Colorless PCs Mean Total			**1063.91±25.44**	**855.93±1.01**	**937.77±7.93**	**951.89±5.21**	**917.95±12.68**	**836.58±12.09**	**830.20±25.15**	**582.17±2.56**

RT: Retention time, ND: Not detected, NA: Not applicable, PCs: Phenolic contents.
LSD Test ($P \leq 0.05$; Levels that are not connected by the same letter are significantly different).
Results are mean of three replications ± standard deviation (SD).
Values with distinct letters (A~ H) in the similar row are significantly different at $P<0.05$.

Table 4.2. Determination of total phenolic compounds in pulps of eight fig (*F.carica* L.) cultivars and genotypes

	No	Identified Compounds	RT	01-1N-58	SARILOP	Y-BOZOVA	31-N-05	BURSA SIYAHI	IZMIR BARDAKCI	01-1M-02	01-1N-51
Identified Colored PCs Anthocyanins	1	Cyanidin 3, 5-diglucoside	16.467	0.71±0.00^{A}	0.16±0.00^{H}	0.23±0.00^{D}	0.19±0.00^{F}	0.18±0.00^{G}	0.26±0.00^{C}	0.20±0.00^{E}	0.32±0.00^{B}
	2	(Epi)catechin-(4-8)- Cyanidin 3-rutinoside	18.173	0.14±0.00^{A}	ND	0.05±0.00^{F}	0.05±0.00^{E}	0.06±0.00^{C}	0.06±0.00^{D}	0.04±0.00^{G}	0.13±0.00^{B}
	3	(Epi)catechin-(4-8)- Cyanidin 3-rutinoside	20.072	0.19±0.00^{A}	ND	0.07±0.00^{F}	0.08±0.00^{E}	0.09±0.00^{D}	0.12±0.00^{C}	0.05±0.00^{G}	0.16±0.00^{B}
	4	Cyanidin 3-glucoside	25.411	2.20±0.00^{A}	0.24±0.00^{G}	0.80±0.00^{D}	0.47±0.00^{E}	0.48±0.00^{E}	1.06±0.00^{C}	0.35±0.01^{F}	1.12±0.00^{B}
	5	Cyanidin 3-rutinoside	27.486	27.86±0.02^{A}	4.35±0.00^{H}	8.02±0.01^{E}	8.10±0.01^{D}	5.60±0.01^{G}	8.62±0.00^{C}	6.53±0.02^{F}	16.93±0.01^{B}
	6	Pelargonidin 3-rutinoside	32.116	0.47±0.00^{A}	0.11±0.00^{E}	0.15±0.00^{D}	0.08±0.00^{F}	0.07±0.00^{F}	0.16±0.00^{C}	0.15±0.01^{D}	0.39±0.00^{B}
	Colored PCs Mean Total			**31.57±0.02**	**4.86**	**9.32±0.01**	**8.98±0.01**	**6.48±0.01**	**10.28**	**7.33±0.04**	**19.04±0.01**
Identified Colorless PCs mg GAE/100 g DW	1	Gallic acid di-pentoside	27.16	11.69±0.00^{AB}	0.21±0.00^{B}	8.88±0.06^{AB}	14.57±0.21^{A}	9.87±13.96^{AB}	18.26±0.20^{A}	18.07±0.17^{A}	11.25±0.04^{AB}
	2	Gallic acid	14.49	0.81±0.06^{AB}	0.61±0.00^{B}	0.91±0.03^{AB}	0.78±0.06^{AB}	0.36±0.00^{B}	0.69±0.69^{B}	1.39±0.08^{A}	0.74±0.00^{B}
	3	Catechin	31.23	14.15±1.50^{E}	11.22±0.72^{F}	18.06±0.70^{D}	13.34±0.91^{EF}	97.09±1.59^{A}	46.30±1.64^{B}	17.59±0.08^{D}	23.04±0.06^{C}
	4	Epicatechin	39.21	116.49±0.06^{A}	2.90±0.08^{F}	10.98±0.28^{DEF}	6.68±0.23^{EF}	14.95±8.86^{D}	39.72±1.36^{B}	14.22±1.55^{DE}	23.31±0.06^{C}
	5	Prenylhydroxygenistein	22.43	149.83±0.46^{B}	158.88±2.80^{A}	138.55±0.49^{C}	66.04±0.37^{F}	90.98±7.09^{E}	20.39±5.84^{G}	63.09±1.99^{F}	108.97±0.29^{D}
	6	Neochlorogenic acid	25.85	164.05±4.77^{C}	203.12±5.15^{A}	188.32±0.34^{B}	6.41±0.59^{H}	117.06±0.92^{E}	19.81±0.91^{G}	69.95±1.85^{F}	134.66±0.36^{D}
	7	Chlorogenic acid	31.64	16.55±0.74^{H}	363.08±0.31^{A}	102.29±2.36^{DE}	133.00±2.86^{B}	93.64±5.99^{F}	116.34±2.14^{C}	111.26±0.63^{CD}	25.38±0.07^{G}
	8	Cryptochlorogenic acid	37.24	0.37±0.02^{D}	1.43±0.04^{BCD}	ND	0.94±1.30^{CD}	1.87±0.12^{BC}	1.19±0.03^{CD}	2.47±0.01^{AB}	3.61±0.05^{A}
	9	Dihydroxybenzoic acid pentoside	24.92	2.26±0.02^{B}	3.10±0.03^{AB}	2.20±0.13^{AB}	2.37±0.00^{AB}	2.13±1.33^{B}	3.51±0.32^{A}	2.77±0.15^{AB}	3.04±0.04^{C}
	10	Luteolin	64.57	0.64±0.01^{C}	0.28±0.00^{D}	0.31±0.00^{D}	0.25±0.00^{D}	1.25±0.04^{B}	0.34±0.00^{D}	1.71±0.07^{A}	1.23±0.07^{B}
	11	Dihydroxybenzoic acid di-pentoside	29.54	5.72±0.03^{A}	6.92±0.19^{A}	2.64±0.05^{B}	3.17±0.21^{B}	3.13±2.61^{B}	4.68±0.21^{AB}	5.38±0.04^{AB}	5.64±0.12^{A}
	12	Apigenin C-hexoside C-pentoside	43.31	1.64±0.02^{DE}	1.60±0.09^{DE}	1.89±0.25^{D}	1.55±0.05^{E}	1.02±0.02^{F}	2.07±0.06^{C}	3.50±0.05^{A}	2.55±0.03^{B}
	13	Quercetin glucoside-O-rutinoside	41.69	1.26±0.01^{CD}	3.27±0.19^{A}	0.99±0.22^{CD}	0.76±0.01^{D}	2.62±0.83^{AB}	1.77±0.27^{BC}	1.67±0.51^{CD}	1.48±0.03^{CD}
	14	Quercetin 3-O-rutinosid (rutin)	47.63	8.10±0.14^{ABC}	4.12±0.05^{C}	2.78±0.29^{C}	4.73±0.06^{BC}	6.74±5.81^{ABC}	5.52±0.20^{BC}	11.83±1.61^{A}	9.95±0.02^{AB}
	15	Hyperoside	48.01	2.75±0.02^{F}	4.28±0.13^{CD}	8.82±0.41^{A}	4.59±0.73^{C}	9.09±0.11^{A}	7.17±0.45^{B}	3.60±0.22^{DE}	3.25±0.10^{EF}
	16	Quercetin 3-O-glucoside (isoquercetin)	49.28	2.73±0.34^{A}	1.88±0.02^{AB}	1.29±0.16^{B}	1.93±0.01^{AB}	2.01±0.34^{AB}	2.47±0.74^{AB}	2.30±1.12^{AB}	2.18±0.13^{AB}
	17	Kaempferol-3-O-rutinoside (Nicotiflorin)	43.83	1.66±0.23^{BC}	2.11±0.40^{BC}	1.83±0.07^{BC}	1.14±0.75^{C}	2.00±1.22^{BC}	2.24±0.10^{ABC}	3.59±0.31^{A}	2.71±0.11^{AB}
	18	Apigenin-6,8-C-diglucoside (Vicenin)	50.54	0.38±0.00^{F}	0.70±0.01^{D}	1.89±1.30^{A}	0.31±0.00^{F}	1.75±0.03^{B}	0.65±0.05^{D}	0.61±0.02^{DE}	0.56±0.09^{E}
	19	Kaempferol 7-O-glucoside	44.29	0.39±0.01^{F}	0.65±0.00^{D}	0.63±0.04^{D}	0.23±0.00^{G}	10.29±0.02^{A}	0.51±0.00^{E}	1.34±0.03^{C}	1.53±0.11^{B}
	20	Isorhamnetin-3-O-rutinoside	45.02	0.62±0.00^{E}	1.27±0.04^{B}	0.59±0.00^{E}	0.43±0.00^{F}	2.31±0.04^{A}	0.43±0.00^{F}	1.06±0.00^{C}	0.87±0.04^{D}
	21	Luteolin C-hexoside C-pentoside 1	40.55	0.31±0.00^{D}	0.19±0.00^{F}	0.10±0.00^{G}	0.44±0.00^{B}	0.81±0.01^{A}	0.35±0.00^{C}	0.26±0.00^{E}	0.30±0.02^{D}
	22	Luteolin C-hexoside C-pentoside 2	46.95	0.61±0.01^{G}	1.58±0.00^{D}	0.76±0.00^{F}	1.32±0.04^{E}	2.57±0.03^{C}	0.56±0.00^{G}	5.41±0.02^{A}	3.03±0.04^{B}
	23	Apigenin 8-C-glucoside (Vitexin)	35.10	3.70±0.18^{B}	3.29±0.01^{C}	4.60±0.03^{A}	0.73±0.01^{G}	1.35±0.04^{F}	4.61±0.00^{A}	1.63±0.01^{E}	1.92±0.00^{D}
	24	Apigenin 6-C-glucoside (Isovitexin)	47.48	7.72±0.07^{C}	4.21±0.07^{F}	3.99±0.03^{G}	7.11±0.03^{D}	1.79±0.09^{H}	5.05±0.04^{E}	10.29±0.03^{A}	9.09±0.13^{B}
	Colorless PCs Mean Total			**514.43±4.89**	**780.90±9.08**	**503.30±1.88**	**272.82±4.42**	**476.68±24.23**	**304.63±6.46**	**355.99±7.89**	**380.29±0.21**

RT: Retention time, ND: Not detected, NA: Not applicable, PCs: Phenolic contents:
LSD Test ($P \leq 0.05$; Levels that are not connected by the same letter are significantly different).
Results are mean of three replications ± standard deviation (SD).
Values with distinct letters (A~ H) in the similar row are significantly different at $P<0.05$.

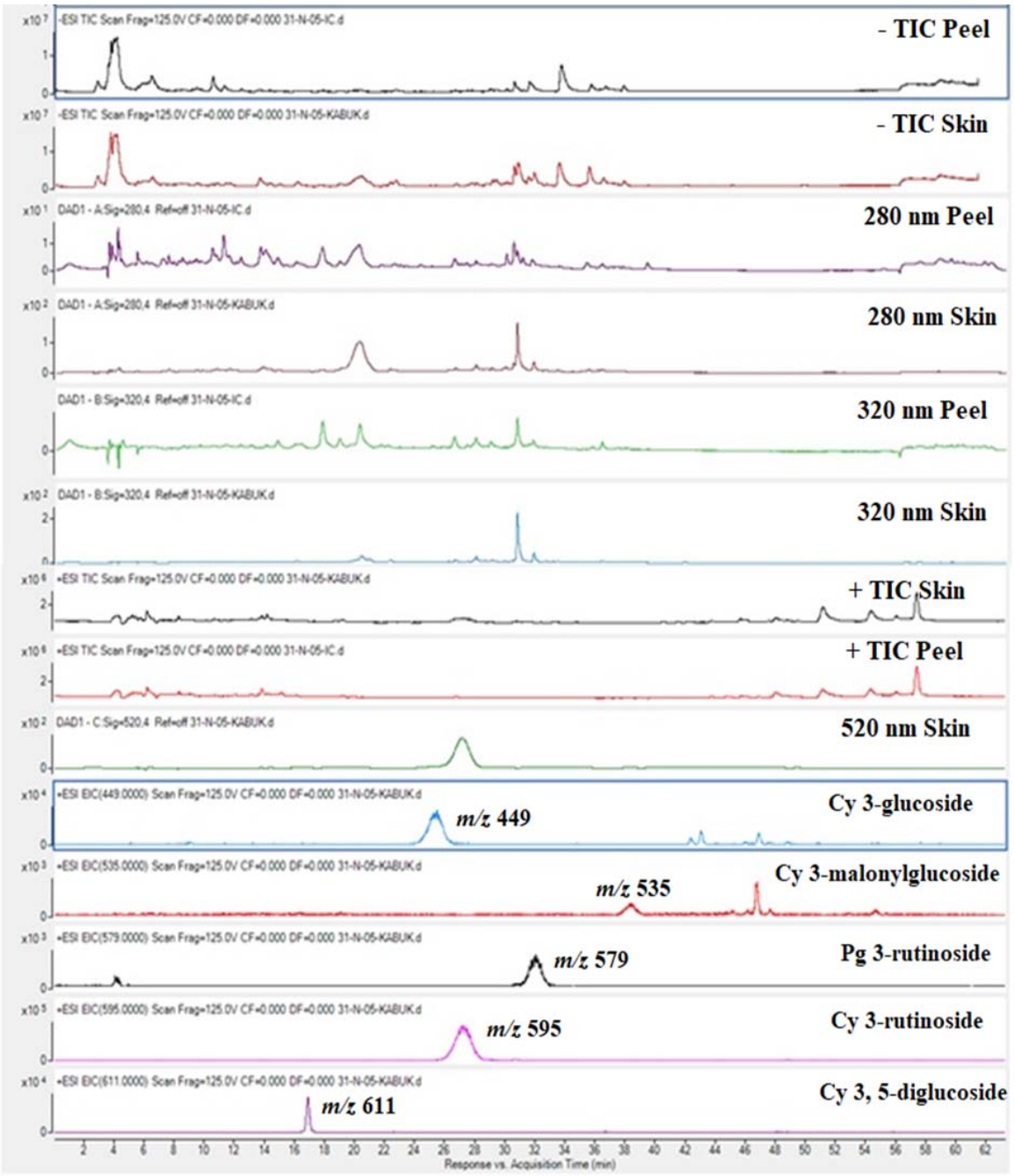


Figure 4.1. Phenolic compounds chromatogram of selected fig cultivars and genotypes (peels and pulps) with their retention times

-TIC: negative total ion count, +TIC: positive total ion, Cy: cyanidin, Pg: pelargonidin.

4.1.1. Anthocyanin pigment contents and their distribution

In the present study, the content and distribution of anthocyanins were analyzed and multiple colors were investigated in both the peels and pulps of eight distinct fig cultivars and genotypes. The experimented 'Sarilop', cultivar illustrates green-yellowish peel color. The ‘’Izmir Bardakci’’ cultivar exhibits a yellowish-green peel color. ‘Yesil Bozova’ showed greenish-light purple peel color and a genotype ‘01-1M-02’ represented greenish-purple peel color. All of the above-mentioned cultivars and genotypes demonstrated a brownish-light-pink pulp color. The

other three genotypes (01-1N-58, 31-N-05, 01-1N-51) and a cultivar (Bursa Siyahi) displayed an intense dark violet-purple peel color and a dark pink pulp color. Significant differences were found in the anthocyanin content between peel, pulp and within cultivars and genotypes.

The individual contents of various identified anthocyanin pigments detected in the peel (Table 4.1) and the pulp (Table 4.2) of *F. carica* L. cultivars and genotypes. The maximum mean value of anthocyanins related to peel was recorded as 151.25 mg/100 g DW in the '01-1N-58' (dark violet purple) genotype whereas, the minimum mean value was noted as 0.23 mg/100 g DW in 'Sarilop' (green-yellowish) cultivar (Table 4.1). Current findings are consistent with previously published studies (Çalişkan and Polat, 2011; Pereira et al., 2017; Zheng et al., 2018; Karantzi et al., 2021; Saleem et al., 2022). These studies documented an increased amount of total anthocyanins in the peels of dark fig varieties 'MM', 'CDN' and 'CE' and less content of PCs detected in their experimented yellow-green fig varieties 'CDB' and 'BB'. In the present study, the other fig genotype (31-N-05) and cultivar (Bursa Siyahi) had a dark purple color and exhibits relatively lesser mean values of anthocyanin as 129.68 mg/100 g DW and 116.40 mg/100 g DW. Whereas, a moderate level of anthocyanin was observed in '01-1N-51' and '01-1M-02' genotypes. Furthermore, 'Yesil Bozova' and 'Izmir Bardakci' (yellowish-green) were among the least anthocyanin-containing cultivars (Table 4.1).

The maximum amount of anthocyanins mean value for pulp was measured as 31.57 mg/100 g DW in '01-1N-58' genotype while the minimum mean value was found as 4.86 mg/100 g DW in 'Sarilop' cultivar (Table 4.2). The relatively lesser mean value of the anthocyanin in pulp was calculated as 19.04 mg/100 g DW in '01-1N-51' genotype (Table 4.2). The cultivars (Yesil Bozova, Izmir Bardakci and Bursa Siyahi) and genotypes (31-N-05 and 01-1M-02) represented a moderate to least anthocyanin level (Table 4.2).

Among the identified anthocyanins, the proportion of cyanidin derivatives was the most abundantly detected in both peel and pulp, (Table 4.1 and Table 4.2). Prior studies investigated the isolation and detection of anthocyanin components in figs and also revealed that cyanidin as well as its derivatives played a major role in the production of fig skin color (Wang et al., 2017; Li et al., 2021).

In our study, two phenolic cyanidin compounds Cy 3-O-rutinoside and Cy 3-glucoside exhibits mean value of 0.20–135 (mg/100 g DW) and 0.03-10.66 (mg/100 g DW) in the peel of all cultivars and genotypes (Table 4.1). Similarly, these two phenolic cyanidin compounds were also the main compound observed in pulp with the mean value of 4.35-27.86 (mg/100 g DW) and 0.24-2.20 (mg/100 g DW), respectively (Table 4.2). Therefore, Cy 3-O-rutinoside was the predominant compound detected in peels and pulps of all cultivars and genotypes followed by Cy 3-glucoside (Table 4.1 and Table 4.2).

Similar findings were also reported in the previous study by Arvaniti et al. (2019). Our findings were also supported by various studies conducted across the globe. They also endorsed

and documented the key presence of cyanidine-3-O-rutinoside and Cy 3-glucoside (colored phenolics) in fig peels and pulps (Ammar et al., 2015; Çalişkan and Polat, 2011; Dueñas et al., 2008; Hssaini et al., 2022; Kamiloglu and Capanoglu, 2015; Karantzi et al., 2021; Palmeira et al., 2019; Singh et al., 2022; Solomon et al., 2006; Trad et al., 2013; Vallejo et al., 2012; Veberic and Mikulic-Petkovsek, 2016; Wang et al., 2017; Wojdyło et al., 2016; Yemiş et al., 2012).

Additionally, our findings showed that Cyanidin 3, 5-diglucoside was present in pulp except 'Sarilop' cultivar (Table 4.2). Cyanidin 3, 5-diglucoside was also found to be more abundant in the skin with a mean value of 0.24-1.71 mg/100 g DW (Table 4.1) than in pulp 0.16-0.71 mg/100 g DW (Table 4.2). Additionally, the value of Pelargonidin 3-0-rutinoside was noted to be high in the peel of the '01-1N-58' genotype, with a mean value of 2.35 mg/100 g DW (Table 4.1), whereas in the pulp of '01-1N-58' it was 0.47 mg/100 g DW (Table 4.2). Although cyanidin 3-malonylglucoside was observed in skin extracts of all the experimental studied cultivars and genotypes except Sarilop and Izmir Bardakci, no shreds of evidence of this compound were detected when the extracts were analyzed in the pulp (Table 4.1and Table 4.2). This study revealed that the fig's skin color plays an important role in the accumulation of phenolics particularly colored PCs like anthocyanins. Moreover, dark colors have a greater appeal to local consumers for their nutritious point of view. A group of compounds called anthocyanins that contribute to the color of fig peels might display these characteristics due to the high amount of anthocyanins, particularly in the ripening stage of the fruit.

4.1.2. Colorless phenolic contents in the peel and pulp of fig (*Ficus carica* L.) cultivars and genotypes

Concentrations and distribution of colorless PCs in peel and pulp of eight Turkish fig cultivars and genotypes were investigated, which were comprised of multiple colors such as green-yellowish, yellowish-green, greenish light purple, and violet dark purple The mean values of the colorless phenolic concentrations varied significantly among pulps and peels, as well as among some of the experimented cultivars and genotypes. A total of 24 colorless phenolics were identified in the peel and pulp of eight fig cultivars and genotypes (Table 4.1 and Table 4.2). The higher amount of the colorless phenolics was computed as 1063.91 mg GAE/100 g DW in the peel of 01-1N-58 genotype, whereas the minimum mean value was found as 582.17 mg GAE/100 g DW in the 01-1N-51 genotype (Table 4.1). Furthermore, in the current study, among the identified colorless phenolics, chlorogenic acid (CA) was found to be the dominant compound in peels of the eight fig cultivars and genotypes, with the total mean values ranging between 91.13-524.74 mg GAE/100 g DW (Table 4.1). Our results were supported by the study conducted by Pereira et al. (2017). Whereas, the other most common compounds detected were epicatechin and quercetin 3-O-rutinoside (rutin) which were reported with mean concentration ranges of 12.06-151.46 and

64.61-104.46 mg GAE/100 g DW, respectively. Current findings are also consistent with a study conducted by Hssaini et al. (2020).

In the present study, we observed that the utmost value was computed as 780.90 mg GAE/100 g DW in Sarilop cultivar, whereas the lowest amount of the colorless phenolics was found to be 272.82 mg GAE/100 g DW in the pulp of the fig genotype 31-N-05 (Table 4.2). Besides, among the detected colorless phenolics, chlorogenic acid (CA) was found to be the dominant compound in the pulp of the eight fig cultivars and genotypes, with the total mean values ranging between 16.55-363.08 mg GAE/100 g DW (Table 4.2). The previous studies carried out by Pereira et al. (2017); Veberic and Mikulic-Petkovsek (2016); Wojdyło et al. (2016) also reported the presence of a higher value of CA in the pulp of fig experimented samples. They also confirmed the higher value of CA in the brown and green-yellow fig variety 'Škofjotka'.

Furthermore, the other key compounds identified in our present study were neochlorogenic acid and prenylhydroxygenistein, which were depicted with mean values ranging from 6.41-203.12 mg GAE/100 g DW and 20.39-158.88 mg GAE/100 g DW, respectively. This present study further revealed the complete dominance of colorless phenolics in peels rather than the pulp of eight experimented fig cultivars and genotypes (Table 4.1 and Table 4.2). These findings were consistent with the prior studies carried out by Çalişkan and Polat (2011); Hssaini et al. (2021); Pereira et al. (2017); Vallejo et al. (2012) and found that PCs content of fruit peels was higher than that of pulp extracts in all their experimented samples.

From a nutritional perspective, the high concentration of phenolics in the peel is particularly interesting. Results of our studies showed that fig variety and color are the main factors responsible for TPC variations. Our findings were also supported by (Khadhraoui et al., 2019; Kebal et al., 2022). It is also recommended to consume whole fruit with a dark color peel to maximize the health benefits of fig fruit due to the antioxidant properties of the anthocyanins (Karantzi et al., 2021; Pereira et al., 2017).

Nonetheless, the previously published research studies reveal a number of other factors which also play a key role in the TPC and bioactive compounds variations like , fruit collection stage, cultural and cultivation practices, climatic changes, geographic origin, various pedo-climatic factors and solvents used in various extraction protocols (Meziant et al., 2021; dos Anjos Cruz et al., 2022). The color difference among the fig varieties and cultivars could be attributed to the expression of genes that control anthocyanin synthesis. Our results observed that dark-colored figs have higher levels of phenolic chemicals. It is most likely related to an increase in anthocyanin pigments (Solomon et al., 2006; Kebal et al., 2022).

4.2. Sugar contents in the peels and pulps of experimented fig (*F.carica* L.) cultivars and genotypes

The flavor of a fruit comprises aroma, flavor, taste and mouth-feel attributes, describing a precise sensory profile that impacts consumer evaluations and determines the quality. It is primarily sugars that give the fruit its edible quality, primarily sweetness, which plays a large role in consumer satisfaction. Based on the sugar content and precise sugar profile (the relative content of each sugar), the sweetness intensity varies as well (Cirilli et al., 2016). Therefore, the constituents and distribution of sugar were analyzed in eight Turkish figs. Various colors, including green-yellowish, yellowish-green, greenish light purple, and violet dark purple were investigated in both the peel and pulp of *F. carica* L. cultivars and genotypes. Significant differences were found in the total sugar content in both pulp and skin and among some cultivars and genotypes. Similarly, a study conducted by Hssaini et al. (2021) regarding 11 fig fruit cultivars, reported the differences in total identified sugar values (sucrose, glucose and fructose) among the cultivars.

The individual concentrations of various identified sugars including, sucrose, glucose, and fructose with the mean values detected in the skin and pulps of the fig cultivars and genotypes (Table 4.3). The comparisons of retention times and spikes of samples with reference compounds led to the identification of chromatographic peaks (Fig. 4.3). The maximum mean values of sugars in the skin were recorded as 504.64 g/ kg−1 DW in ‘Izmir Bardakci’ (yellowish-green) cultivar and minimum mean value was found as 380.25 g/ kg−1 DW in ‘Bursa Siyahi’ (dark violet purple) cultivar (Table 3). Compare to the highest sugar mean value, our results showed that the ‘Yesil Bozova’ (greenish light purple) cultivar, ‘31-N-05’ (Dark Purple), ‘01-1M-02’ (greenish-purple) and ‘01-1N-51’ (violet purple) genotypes exhibit the lesser mean values of 497.68, 502.84, 496.82 and 496.53 g/ kg−1 DW (Table 4.3). Whereas, the ‘Sarilop’ (green-yellowish) cultivar and ‘01-1N-58’ (intense violet purple) genotype had a moderate level of total sugars, with mean values of 475.95 g/ kg−1 DW and 474.98 g/ kg−1 DW, respectively (Table 4.3).

Table 4.3. Analysis of total sugars and antioxidant capacity in peels and pulps of eight figs (*F.carica* L.) cultivars and genotypes

	Measured Parameters	01-1N-58	SARILOP	Y-BOZOVA	31-N-05	BURSA SIYAHI	IZMIR BARDAKCI	01-1M-02	01-1N-51
	Fig Peels								
Sugar Composition (g/ kg−1 DW)	Sucrose	56.41±0.19^{D}	45.12±0.14^{F}	42.37±0.46^{G}	55.69±0.23^{E}	23.50±0.02^{H}	69.71±0.32^{B}	60.08±0.28^{C}	80.62±0.76^{A}
	Glucose	222.09±0.47^{D}	219.93±0.24^{E}	226.50±0.11^{B}	223.21±0.39^{C}	183.24±0.24^{F}	222.39±0.10^{D}	238.79±0.18^{A}	226.85±0.13^{B}
	Fructose	196.48±0.49^{F}	210.90±0.11^{D}	228.81±0.98^{A}	223.94±0.37^{B}	173.52±0.41^{H}	212.54±0.57^{C}	197.96±0.14^{E}	189.06±0.91^{G}
	Mean Total	**474.98±1.15**	**475.95±0.49**	**497.68±1.55**	**502.84±0.99**	**380.25±0.67**	**504.64±0.99**	**496.82±0.60**	**496.53±1.8**
Antioxidant Capacities mM Trolox /gDW	DPPH	28.08±0.04^{A}	7.84±0.35^{J}	21.47±0.99^{C}	28.05±0.37^{A}	26.64±0.76^{B}	9.59±0.53^{F}	19.37±0.95^{D}	14.05±0.62^{E}
	ABTS	124.62±0.69^{B}	43.81±0.29^{H}	89.7±0.23^{D}	127.34±1.15^{A}	124.61±1.08^{B}	52.55±1.81^{F}	96.59±1.36^{C}	76.55±2.01^{E}
	Mean Total	**149.7±0.73**	**51.65±0.64**	**111.17±1.22**	**155.39±1.52**	**151.25±1.84**	**62.14±2.34**	**115.96±2.31**	**90.6±2.63**
	Fig Pulps								
Identified Sugars (g/ kg−1 DW)	Sucrose	58.48±0.76^{A}	45.33±0.58^{D}	28.07±0.33^{F}	46.45±0.25^{C}	45.39±0.23^{D}	46.46±0.15^{C}	53.74±0.23^{B}	41.76±0.23^{E}
	Glucose	227.59±0.46^{F}	236.48±0.51^{D}	225.86±0.70^{G}	237.37±0.31^{C}	250.16±0.31^{A}	217.53±0.10^{H}	248.86±0.10^{B}	230.26±0.33^{E}
	Fructose	225.42±0.73^{G}	249.62±0.83^{D}	270.33±1.02^{A}	255.50±0.61^{B}	251.38±0.58^{C}	229.46±0.41^{F}	237.26±1.04^{E}	236.86±0.48^{E}
	Mean Total	**511.49±1.95**	**531.43±1.92**	**524.26±2.05**	**539.31±1.17**	**546.94±1.12**	**493.45±0.66**	**539.86±1.37**	**508.87±1.04**
Antioxidant Capacities mM Trolox /gDW	DPPH	9.41±0.14^{F}	5.88±0.31^{H}	5.86±0.33^{H}	4.55±0.28^{I}	6.96±0.34^{G}	5.7±0.37^{H}	6.41±0.4^{G,H}	6.97±0.42^{G}
	ABTS	50.94±0.73^{F}	39.96±0.47^{I}	37.94±0.22^{J,K}	28.45±0.1^{L}	49±0.21^{G}	36.96±1.97^{K}	39.27±1.09^{I,J}	40.85±0.86^{I}
	Mean Total	**60.35±0.87**	**45.84±0.78**	**43.8±0.55**	**33±0.38**	**55.96±0.55**	**42.66±2.34**	**45.68±1.49**	**47.82±1.28**

DW: Dry weight, DPPH: 2, 2′-diphenyl-1-picrylhydrazyl radical, ABTS: 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid.
LSD Test ($P \leq 0.05$; Levels that are not connected by the same letter are significantly different).
Results are mean of three replications ± standard deviation (SD).
Values with distinct letters (A~ L) in the similar row are significantly different at $P<0.05$.

Comparisons of retention times and spikes of samples with reference compounds led to the identification of chromatographic peaks (Figure 4.2).

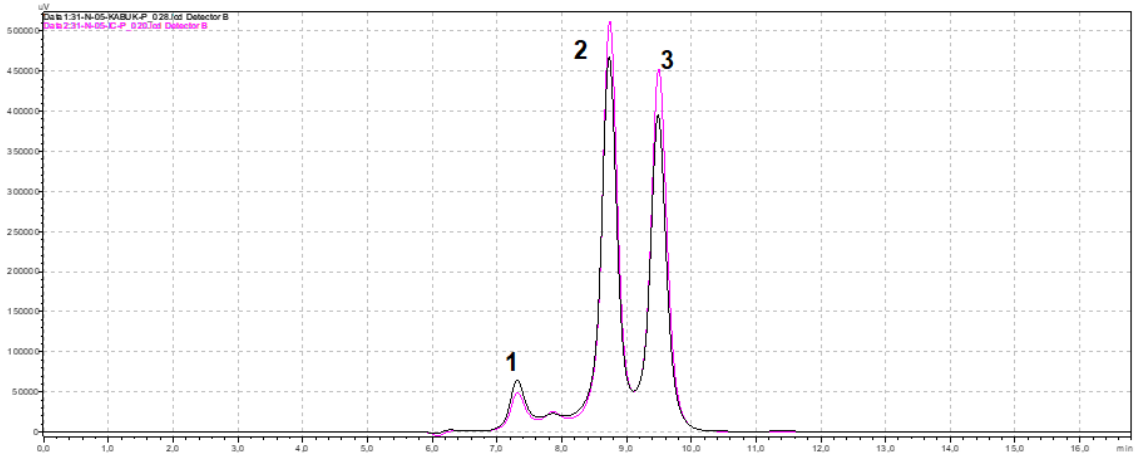


Figure 4.2. HPLC chromatogram of sugars, whereas, 1 indicates (sucrose), 2 (glucose) and 3 (fructose).

The highest mean value of total sugars in pulp was documented as 546.94 g/ kg−1 DW in the 'Bursa Siyahi' cultivar and the lowest mean value was found as 493.45 g/ kg−1 DW in the 'Izmir Bardakci' cultivar. In comparison to the maximum mean value in 'Bursa Siyahi' among the other fig genotypes and cultivars, total sugar contents in pulp revealed that the '01-1M-02' (greenish-purple), '31-N-05' (dark purple), 'Sarilop' (green-yellowish), 'Yesil Bozova' (greenish light purple), '01-1N-58' (intense violet purple) and '01-1N-51' (violet purple) exhibits relatively moderate to lesser mean values of total sugars 539.86, 539.31, 531.43, 524.26, 511.49, and 508.87 g/ kg−1 DW, respectively (Table 4.3).

Of the total sugar contents, glucose was the most abundantly found sugar in the skin, followed by fructose and sucrose. Glucose was the predominant sugar with a mean value of 183.24–238.79 g/ kg−1 DW, followed by fructose (173.52–228.81 g/ kg−1 DW) and sucrose (23.50–80.62 g/ kg−1 DW) (Table 4.3). Current findings are consistent with the study carried out by Palmeira et al. (2019) which observed that glucose is the predominant sugar present in the peel of fig cultivar "Pingo de Mel" (21.0g/100g FW) followed by fructose and sucrose, respectively. Similarly, Wojdyło et al. (2016) also reported the presence of glucose and fructose as the major reducing sugars in 10 of their fig fruits. Furthermore, our findings are supported by other studies (De Souza et al., 2013; Pereira et al., 2017).

In the pulp of fig cultivars and genotypes among the total sugars, fructose was the most frequently observed sugar with a mean value of 225.42-270.33 g/ kg−1 DW, accompanied by glucose 217.53-250.16/ g kg−1 DW and sucrose 28.07-58.48 g/ kg−1 DW. The current study results are supported by other studies performed by Hssaini et al. (2021); Palmeira et al. (2019); Slatnar et al. (2011); Veberic and Mikulic-Petkovsek, (2016); Wojdyło et al. (2016) those reported

the detection of lower value of sucrose in all their experimented fig cultivars. Moreover, the higher value of fructose and glucose in the pulp of fig cultivars results in our study are aligned with the prior study conducted by Wojdyło et al. (2016). They documented their higher fructose and glucose values in two Spanish fig cultivars, namely ‘Coral’ and ‘Verdal’.

4.3. Total antioxidant capacity in the peel and pulp of fig cultivars and genotypes

The ABTS assay is used to evaluate a sample's antioxidant activity based on radical cation ABTS'+ scavenging capacity. In this method, we employed Trolox (a water-soluble version of vitamin E) as a standard and which is commonly used to assess antioxidant activity. Whereas, the DPPH radical scavenging test is also a simple and rapid method of assessing antioxidant activity by assessing the absorbance of a solution following the blend of antioxidant compositions with DPPH radicals (Dong et al., 2019). Therefore, eight distinct Turkish fig cultivars and genotypes have been examined for antioxidant capacity assay. The two assays 2, 2′-diphenyl-1-picrylhydrazyl radical (DPPH) and 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were determined to assess the antioxidant activity in both their peel and pulp for various colors, including green-yellowish, yellowish-green, greenish light purple and violet dark purple. There have been substantial variations observed in the antioxidant contents of peels and pulps, as well as among some cultivars and genotypes.

For validation, similar DPPH and ABTS assays were performed by Sertkaya et al. (2021) on two Turkish commercial fig varieties, ‘Sarilop’ and ‘Bursa siyahi’. Similarly, Aljane et al. (2020) conducted the same experiment on 27 Tunisian Fig ecotypes. The current study, showed that fig genotype ‘01-1N-58’ had the highest antioxidant capacity with mean values measured by DPPH (28.08 mM Trolox /gDW) while ‘31-N-05’ genotype had the highest ABTS value of (127.34 mM Trolox /gDW) (Table 4.3). In contrast, ‘SARILOP’cultivar had the lowest antioxidant capacity measured by DPPH (7.84 mM Trolox /gDW) and ABTS (43.81 mM Trolox /gDW) (Table 4.3). Overall, the high antioxidant capacity (DPPH and ABTS) value (155.39 mM Trolox /gDW) was observed on ‘31-N-05’ genotype and the lowest antioxidant capacity (DPPH and ABTS) value (51.65 mM Trolox /gDW) was noted on ‘SARILOP’ peels (Table 4.3).

Our study illustrates that fig genotype ‘01-1N-58’ had the highest antioxidant capacity measured by DPPH (9.41 mM Trolox /gDW) and by ABTS (50.94 mM Trolox /gDW). However, ‘31-N-05’ genotype had the lowest antioxidant capacity measured by DPPH (4.55 mM Trolox /gDW) and ABTS (28.45mM Trolox /gDW). Overall, the high antioxidant capacity (DPPH and ABTS) value (60.35 mM Trolox /gDW) was observed for ’01-1N-58’ and the lowest antioxidant capacity (DPPH and ABTS) value (33.00 mM Trolox /gDW) was noted for '31-N-05' genotype.

The total DPPH content mean values were measured as 7.84-28.08 mM Trolox /gDW in peels and 4.55-9.41 mM Trolox /gDW in the pulp. These total DPPH assay findings were also revealed by Aljane et al. (2020); Hssaini et al. (2019); Hssaini et al. (2021); Manian et al. (2008);

Oliveira et al. (2010). The total ABTS values were recorded as (43.81-127.3 mM Trolox /gDW) in peels and (28.45-50.94 mM Trolox /gDW) in pulps. Our study also observed that the ABTS method showed a higher antioxidant activity than DPPH method in all samples. According to Sertkaya et al. (2021), fig fruits have shown higher absorbance in the ABTS method than the DPPH method due to hydrophilic compounds in the fruit. Aljane et al. (2020) and Sertkaya et al. (2021) also reported higher values of ABTS over DPPH in their two experimented samples. Additionally, our total ABTS assay results were similar to those reported by Aljane et al. (2020); Hssaini et al. (2019); Hssaini et al. (2021); Solomon et al. (2006).

The total antioxidant values showed that the peel had more antioxidant capacity (DPPH and ABTS) compared to the pulp of the fruit. Moreover, the dark purple-colored fig genotypes (‘01-1N-58’ and ‘31-N-05’) exhibited the maximum antioxidant activity as compared to the yellow-to-green fig cultivar "SARILOP". These results are aligned with the other studies conducted by Aljane et al. (2020); Hoxha and Kongoli, (2016) and Sertkaya et al. (2021). The skin of fig fruit was also found to have more antioxidant activity than the pulp by approximately two to ten times in a study performed by Saleem et al. (2022).

4.4. Principal component analysis (PCA)

The principal component analysis (PCA) model was used to investigate the key variables between the colored anthocyanins and colorless phenolic compounds of studied fig (F. carica L.) genotypes (Figure 4.3 and Figure 4.4). A total of 16 observations (cultivars and genotypes with peels and pulps) have 7 variables (colored phenolics) and 16 observations (peels and pulps) with 24 variables (colorless phenolics) were used (Figure 4.3 and Figure 4.4).

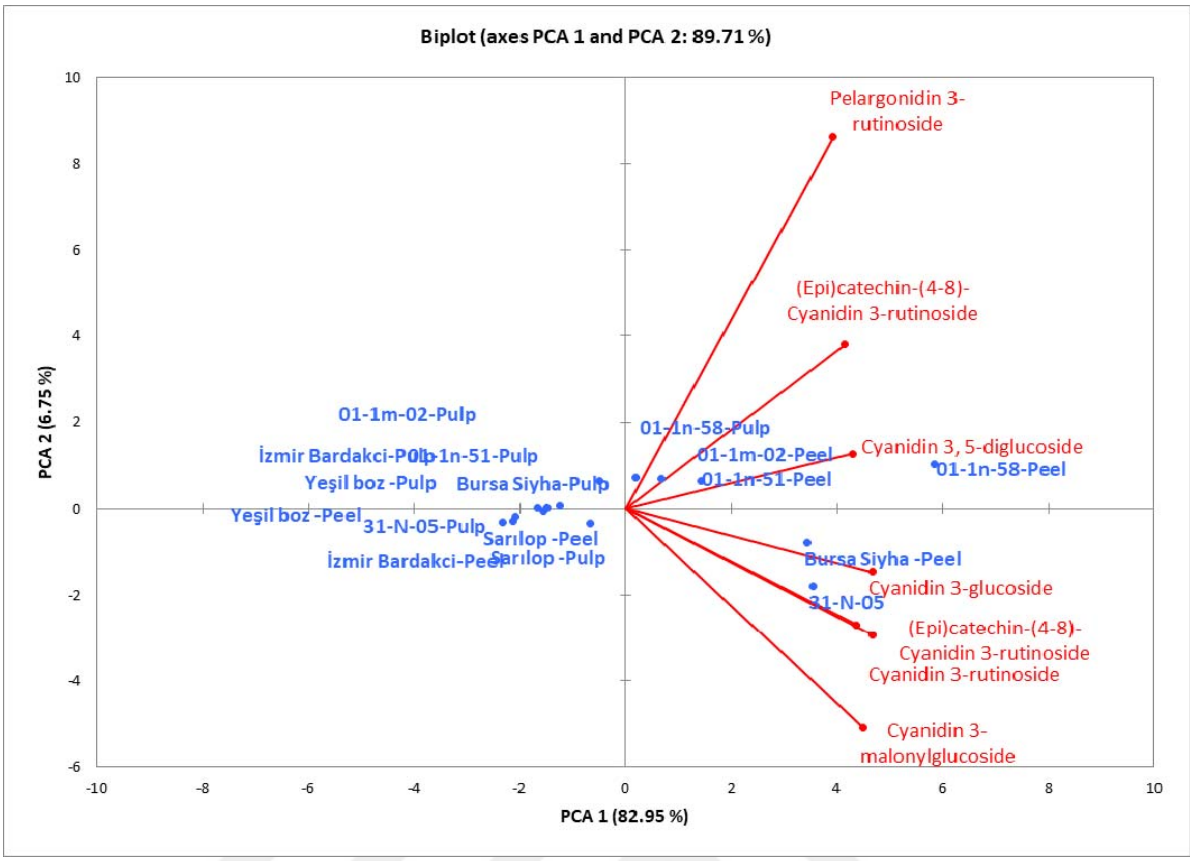


Figure 4.3. Score plot represents the Principal Component Analysis (PCA) of colored phenolic compounds of fig samples (peels and pulps).

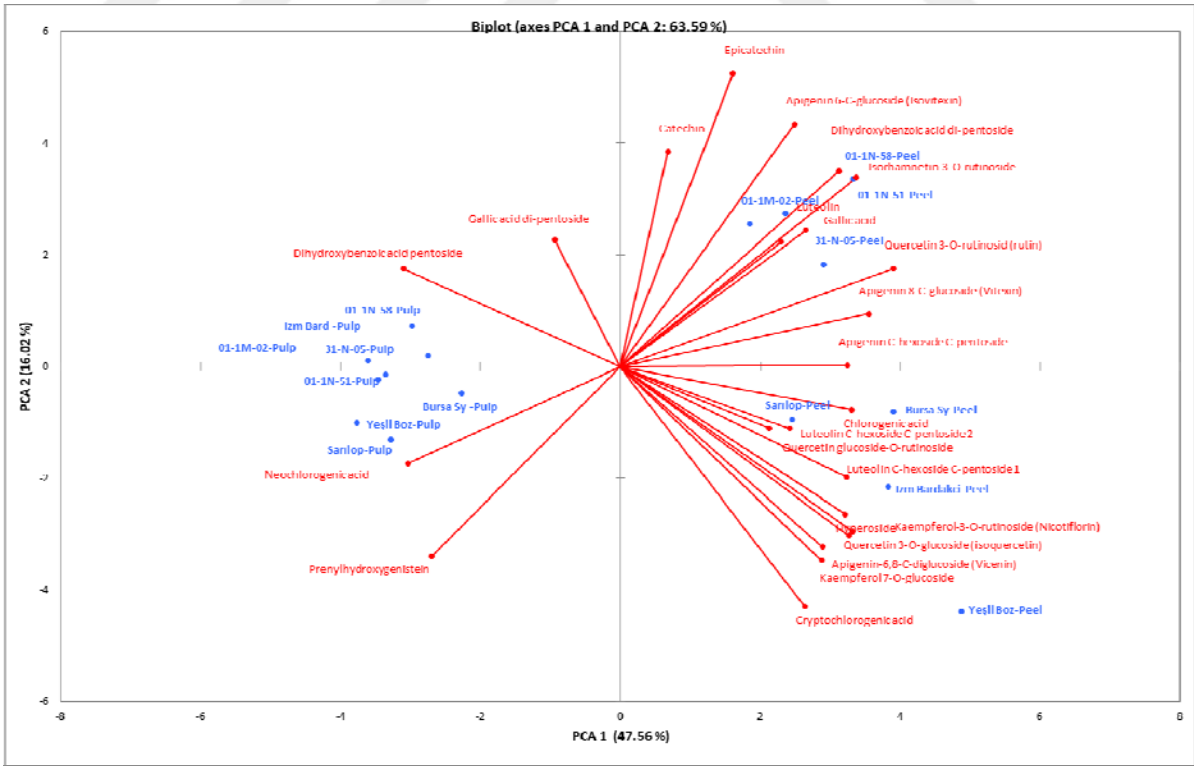


Figure 4.4. Score plot represents the Principal Component Analysis (PCA) of colorless phenolic compounds of fig samples (peels and pulps).

Two principal components explain 89.71 % of the overall variance 82.95 % and 6.75 % for PC1 and PC2, respectively (Figure 4.3). First principal component (PC1), which accounts for 82.95 % of the total variance is determined with the cyanidin 3, 5-diglucoside, (Epi) catechin-(4-8)-cyanidin-3-rutinoside, Pelargonidin-3-rutinoside, cyanidin 3- rutinoside, cyanidin 3- glucoside and cyanidin 3- malonylglucoside. The factors that contributed the most to PC1 (on the positive side) were cyanidin 3, 5-diglucoside and (epi) catechin - (4-8)-cyanidin 3-rutinoside. However, the major contributors to PC2 (negative sides) were Cyanidin 3- rutinoside and Cyanidin 3- glucoside whereas Pelargonidin-3-rutinoside belonged to the positive side, (Figure 4.3). Therefore, it was demonstrated that the genotypes 01-1N-58 (peel), 01-1N-58 (pulp), 31-N-05 and Bursa Siyahi cultivar (peel) contains a higher content of colored anthocyanin polyphenolic compounds (Figure 4.3).

Two principal components explain 63.59 % of the overall variance (47.56 % and 16.02 % for PC1 and PC2, respectively (Figure 4.4). The factors that contributed the most to PC1 (on the positive side) were quercetin 3-O-rutinoside, apigenin 8-C-glucoside and isohamnetin-3-O-rutinoside, while neochlorogenic acid and prenylhydroxygenistein were present at the negative side. In contrast, the key contributors to PC2 (on the negative side) were chlorogenic acid, luteolin C-hexoside C-pentoside 1, hyperoside and quercetin 3-O-glucoside presented on the positive side (Figure 4.4). Thus, it was determined that the genotype 01-1N-58 (peel), 01-1N-51 (peel), and cultivars Yesil Bozova (peel), Bursa Siyahi (peel) and Izmir Bardakci (peel) contain the higher content of colorless phenolic compounds (Figure 4.4).

4.5. Molecular analysis

4.5.1. RNA isolation

For the molecular analyses, RNA isolation was carried out on the two figs, namely ‘01-1N-58’ and 'Sarilop'. Peels of fig samples were used for the RNA isolation. The RNA quality and quantity were determined by a Nanodrop spectrophotometer. The RNA quality and quantity were determined by a Nanodrop spectrophotometer. Additionally, the RNA integrity was confirmed by loading samples on Agarose gel. As a result of Agarose gel electrophoresis, the bands were obtained and shown in (Figure 4.5). The values obtained after the Nanodrop measurements are shown in (Table 4.4).

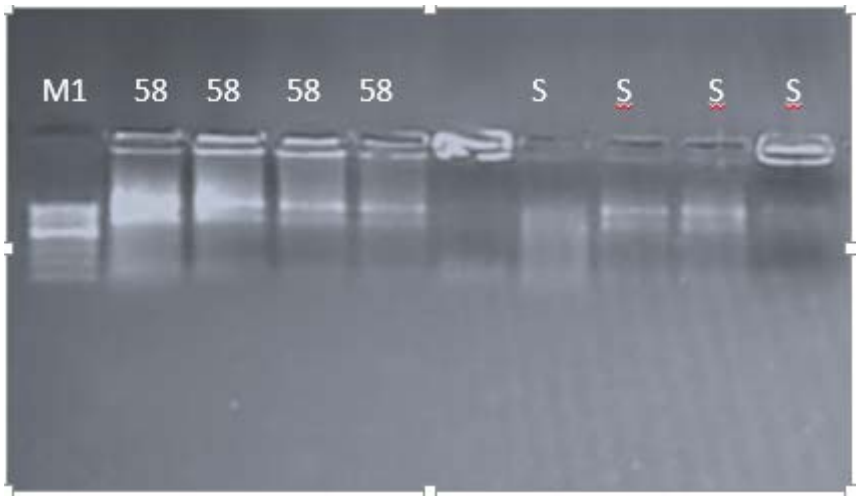


Figure 4.5. Agrose gel bands for RNA isolation from the two fig samples used in the study. M1: 1kb DNA Marker, 58: '01-1N-58', S: 'Sarilop'

Table 4.4. The RNA samples concentration obtained in the study via Nanodrop

Samples	ng/µL	260/280	260/230
01-1N-58	286.1	2.19	2.15
01-1N-58	373.7	2.02	2.36
01-1N-58	448.2	1.96	2.03
01-1N-58	475.6	2.10	2.34
Sarilop	380.3	2.19	2.15
Sarilop	382.5	2.15	2.05
Sarilop	497.6	2.0	2.14
Sarilop	283.7	2.20	2.24

4.6. RNA sequencing analyzes

Based on the total anthocyanin content and the peel color of the two Turkish figs, namely '01-1N-58' (dark-purple) and 'Sarilop (greenish-yellow)," an RNA-Seq study was carried out for the detection of genes associated with anthocyanin using the Illumina NovaSeq 6000™. The lack of gene sequence information hampered the genetic study on fig coloring. Next generation sequencing technology is an efficient and frequent way for obtaining huge genomes, particularly for plants lacking a reference genome (Marioni et al., 2008). Fig fruits have a high concentration of anthocyanins, which is an important characteristic for determining fresh-market fruit quality. However, the molecular process underlying the anthocyanin buildup in fig is still unknown (Li et al., 2021). Therefore, we sequenced two cultivars of figs with significant color differences to enhance DEGs, particularly anthocyanin-associated genes. Therefore, the dark purple and greenish-yellow-colored figs cultivars were selected to do the transcriptomic analysis.

4.6.1. Library construction, de novo assembly, and annotation

In order to identify anthocyanin-related genes, libraries were constructed for two fig samples '01-1N-58' and 'Sarilop'. The total number of raw reads generated in each library ranged from 36,538,618 to 49,360,183 with a Q20 percentage of about 97.94% and a GC percentage of about 58.91% (Table 4.5). These findings suggested that the sequencing data was adequate for a precise de novo assembly. We obtained 39,430,05 (9.3%) unigenes, and 38,325,911 (90.7%) duplicate genes with an average size of 5 M in '01-1N-58' genotype. While, we obtained 43,348,63 (8.9%) unigenes, and 44,608,828 (91.1%) duplicate genes with an average size of 4.7 M in 'Sarilop' cultivar (Figure 4.6).

Similarly, Li et al. (2020) conducted a transcriptomic study on the peels of *Ficus carica* L. They investigated the yellow and red stages based on their colors for anthocyanin biosynthesis. They constructed the two libraries. In each library, the total raw reads generated ranged from 48,269,776 to 55,542,173, with a Q20 percentage of over 97% and 47% of GC. They obtained 186,048 unigenes from the two libraries. Wang et al. (2017) carried a transcriptomic analysis on green peel and purple peel cultivar of fig (*Ficus carica* L.). The total clean reads generated from each libraries were between 25,146,641- 31,591,009 and 79,355 unigenes were gathered. Wang et al. (2021) performed a transcriptomic analysis on the fig variety 'Zibao'. They generated 6,747,389,564 and 9,137,841,606 clean data for control group and bagged flower libraries. Globally some other studies were conducted on the transcriptomic analysis of figs (*Ficus carica* L.) and performed RNA-Seq analysis as well (Cui et al., 2019; Wang et al., 2019; Song et al., 2021).

Table 4.5. The total number of sequences obtained from '01-1N-58' and 'Sarilop' cultivars as a result of RNA-Seq analyzes

Rep	Sample Name	Num Seqs	Sum Len	Read Length	Sum gap	Q20 (%)	Q30 (%)	GC (%)
1	58	42,268,916	5,598,628,561	150	0	97.73	94.31	56.72
2	58	42,268,916	5,602,691,161	150	0	97.32	93.14	56.13
3	58	36,538,618	5,003,498,801	150	0	96.52	91.39	58.66
4	58	36,538,618	4,999,171,843	150	0	97.39	93.13	57.93
5	58	41,208,319	5,462,886,933	150	0	97.48	93.89	58.91
6	58	41,208,319	5,465,439,685	150	0	97.78	93.97	58.32
1	Sarilop	49,360,183	6,809,713,481	150	0	96.52	91.29	58.34
2	Sarilop	49,360,183	6,803,007,839	150	0	97.38	93.08	57.13
3	Sarilop	37,423,053	4,963,346,945	150	0	97.94	94.28	53.48
4	Sarilop	37,423,053	4,962,318,169	150	0	97.5	93.45	53.23
5	Sarilop-	48,943,691	6,678,598,644	150	0	97.56	93.74	56.98
6	Sarilop	48,943,691	6,678,416,369	150	0	96.83	91.99	56.18

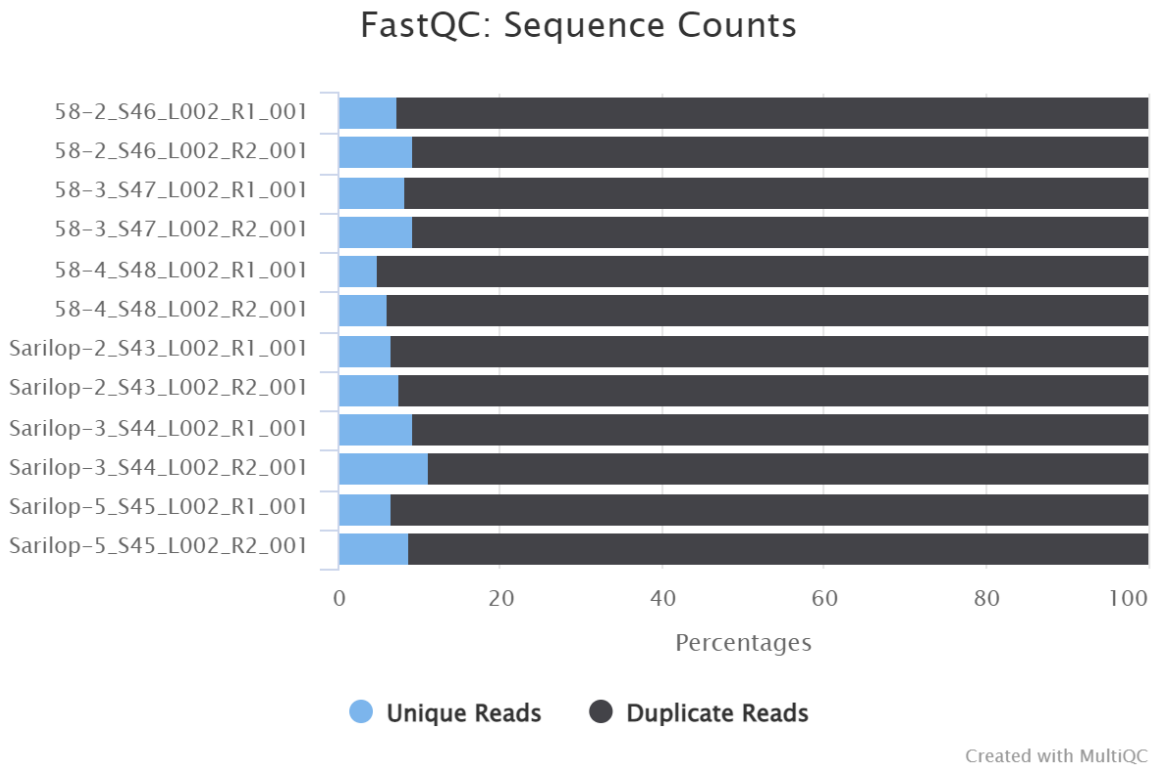


Figure 4.6. Fast QC; sequence counts of '01-1N-58' genotype and 'Sarilop' cultivar

4.6.2. Analysis of genes with different levels of expression (DEGs)

A total of 21102 differentially expressed genes were identified in the libraries of two fig samples '01-1N-58' and 'Sarilop' including 3542 up-regulated and 3164 down-regulated genes (Figure 4.7). A 100% up-regulation was seen in Sarilop cultivar while in 01-1N-58 genotype the up-regulation of expressed genes were recorded as 92.71%. Whereas the 89.32 % of down-regulation was seen in 01-1N-58 genotype and 86.44 % down-regulation was noted on Sarilop fig cultivar (Figure 4.8). Li et al. (2020) conducted a transcriptomic study on the peels of *Ficus carica* L yellow and red stages for anthocyanin biosynthesis. Comparison to the Y library, the R library had expressed 6224 DEGs with 58.15% up-regulated and 41.85% down-regulated genes.

Wang et al. (2017) carried a transcriptomic analysis on green peel and purple peel cultivar of fig (*Ficus carica* L.). As far as the two cultivars of figs are concerned, 1,009 and 616 genes were found to be up-regulated, while 1,376 and 471 genes were noted as down-regulated in the experimented samples. Wang et al. (2021) performed a transcriptomic study on the fig variety of 'Zibao' control group and bagged flower. An up-regulation of genes were seen higher then down-regulatory genes. The up-regulation of genes found in CG was 1208 and 1181 in BF. Cui et al. (2019) conducted a study on common fig and compared their commercial and tree ripening stages. DEGG analysis revealed that the total 1274 genes were recorded as up-regulated and 813 genes were found to be down-regulated in both stages.

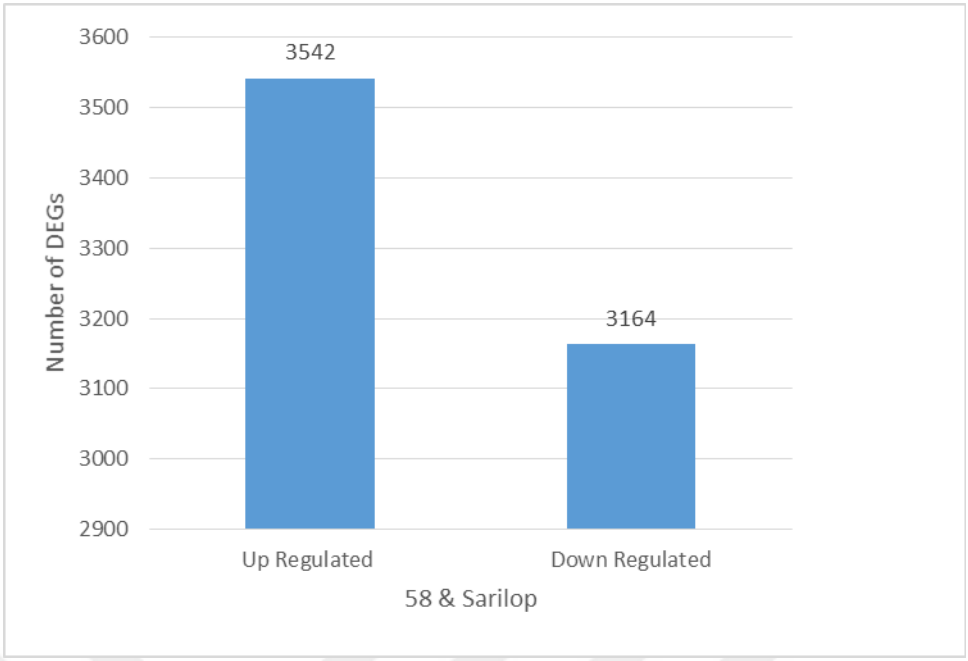


Figure 4.7. Number of DEGs expressed differently between '01-1N-58' genotype and 'Sarilop' cultivar

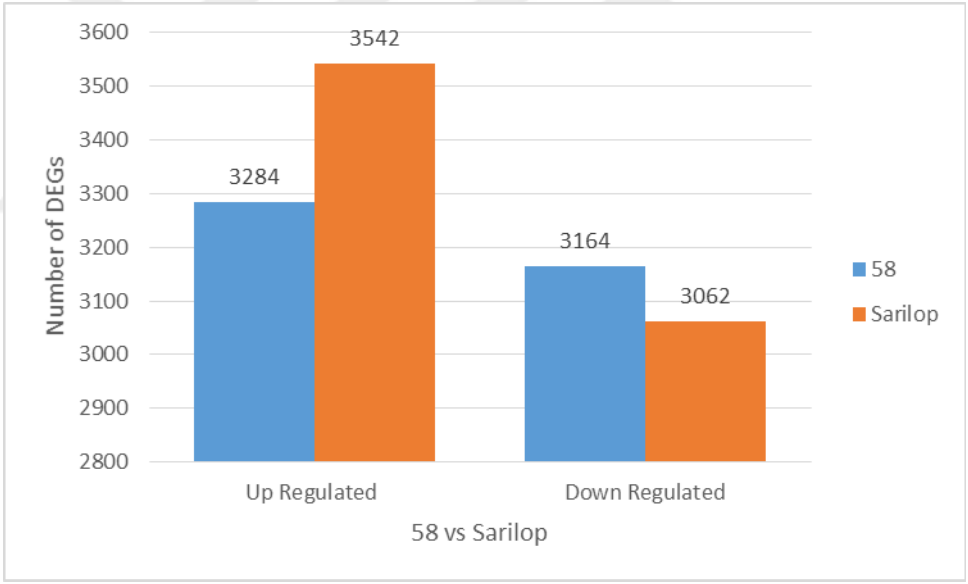


Figure 4.8. Number of DEGs expressed and comparative analysis between '01-1N-58' genotype and 'Sarilop' cultivar

4.6.3. Ontology (GO) and (KEGG) analyzes of genes expressed at different levels

To obtain a comprehensive understanding of DEGs, gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) -based functional enrichment were conducted. According to GO assignments, DEGs were divided into three main categories: biological process, cellular component, and molecular function (Figure 4.13). The dominant subcategories within the biological process category were 'cellular response to chemical stimulus' with 330 nGenes and 'response to oxygen-containing compound' with 325 nGenes. Both the dominant categories in BP showed 1.6 fold enrichment value (Figure 4.9). Among the cellular component category,

‘bounding membrane of organelle’ with 275 nGenes and ‘golgi apparatus’ with 275 nGenes were the two most abundant classes having 1.6 fold enrichment value (Figure 4.10). As far as molecular function category is concerned, ‘transferase activity, transferring phosphorous-containing groups’ with 304 nGenes and ‘kinase activity’ were found to be the two most significant classes having 1.4 fold enrichment value (Figure 4.11).

Among the KEGG pathway analysis, ‘biosynthesis of secondary metabolites’ represented the top enriched KEGG pathways with 233 nGenes having 1.5 fold enrichment value (Figure 4.12). Li et al. (2020) conducted a (GO and KEGG) functional enrichment analysis. GO assignments comprised of BP, CC and MF. Among the biological process the dominant categories were found to be the ‘metabolic process’ and ‘single organism process’ with a percentage of 63.12% and 50.64% respectively. While in the molecular function category ‘ion binding’ and ‘catalytic activity’ were the prominent ones. As far as KEGG pathway analysis was concerned, biosynthesis of secondary metabolites was found to be dominant.

Wang et al. (2017) conducted a (GO and KEGG) functional enrichment analysis. Unigenes with values of 46,748, 34,527, and 22,307 were categorized as BP, CC and MF. The results of KEGG analysis in green and purple fig samples depicted the particular pathways involved were alpha-linolenic acid metabolism, phenylpropanoid biosynthesis plant hormone signal transduction and starch and sucrose. Wang et al. (2021) performed a GO annotation of DEGs and found (1154) unigenes in BP, (674) CC and (572) MF. The results further demonstrated the dominant presence of plant-pathogens interactions pathways, endoplasmic reticulum and plant hormone signal transduction in KEGG pathway. Cui et al. (2019) conducted a GO annotation of DEGs and found (20) MF (17) unigenes in BP and (13) was seen in CC. KEGG pathways showing the significant changes were secondary metabolite biosynthesis.

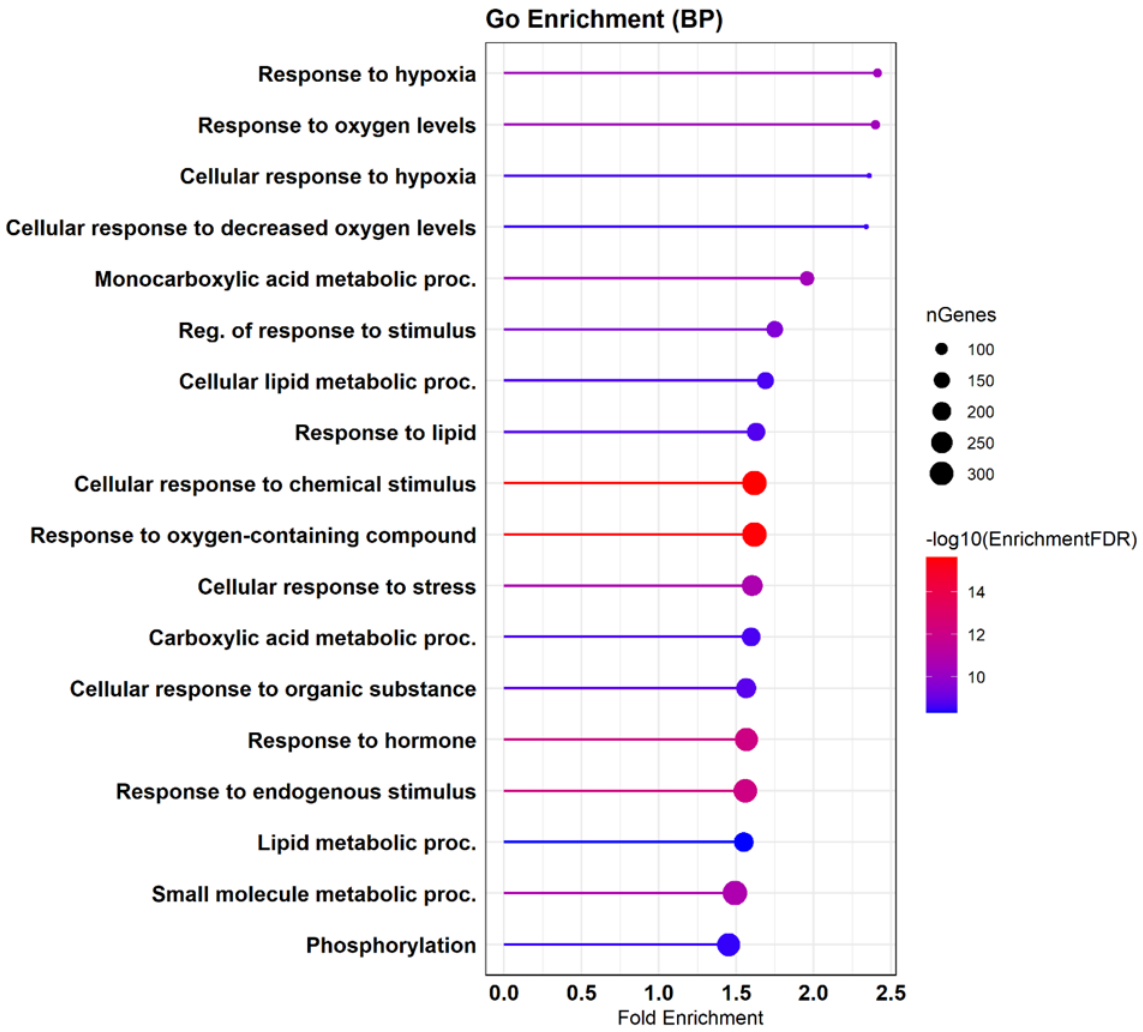


Figure 4.9. Gene ontology biological process between ‘01-1N-58’ genotype and ‘Sarilop’ cultivar

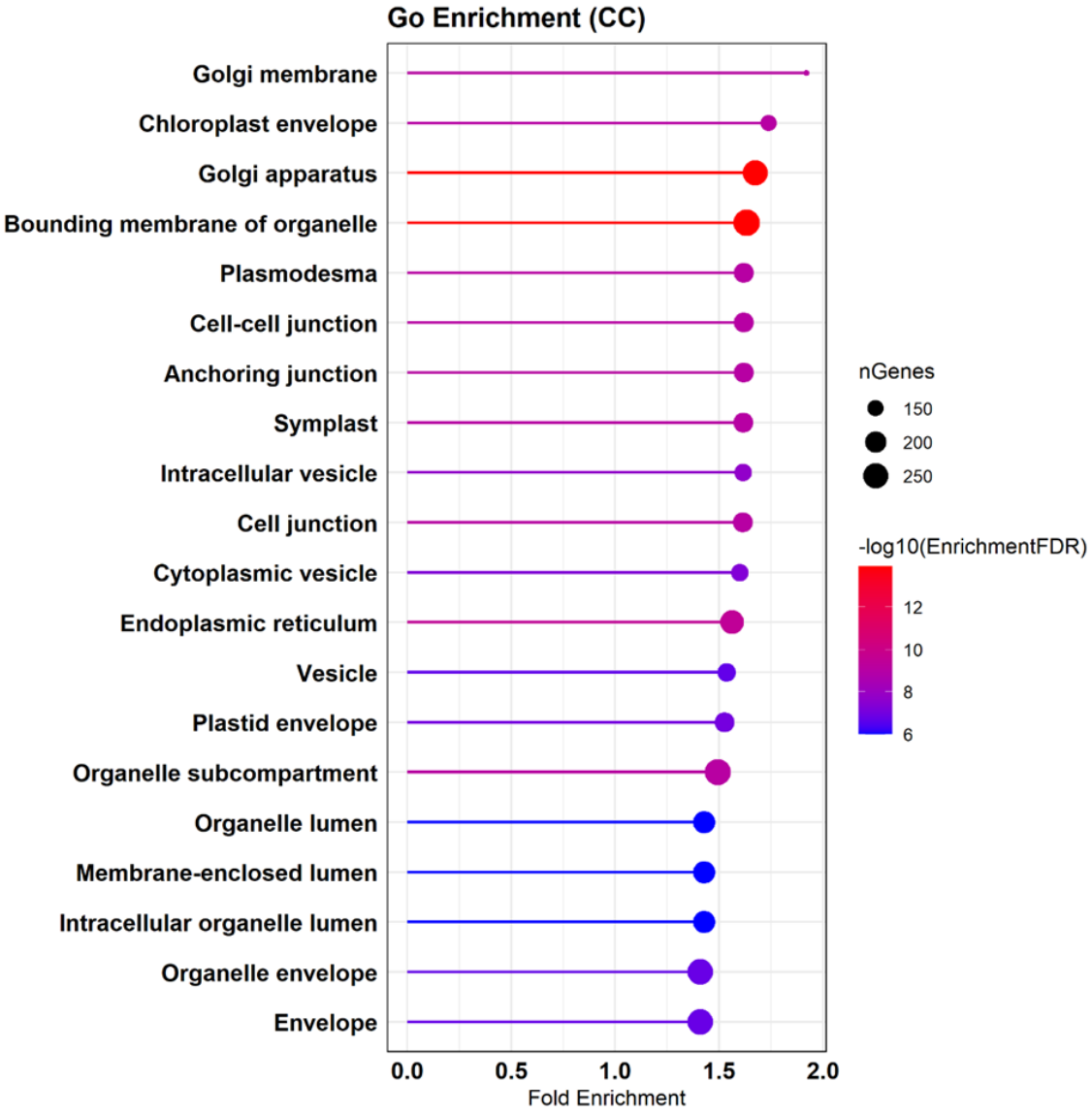


Figure 4.10. Gene ontology cellular component between '01-1N-58' genotype and 'Sarilop' cultivar

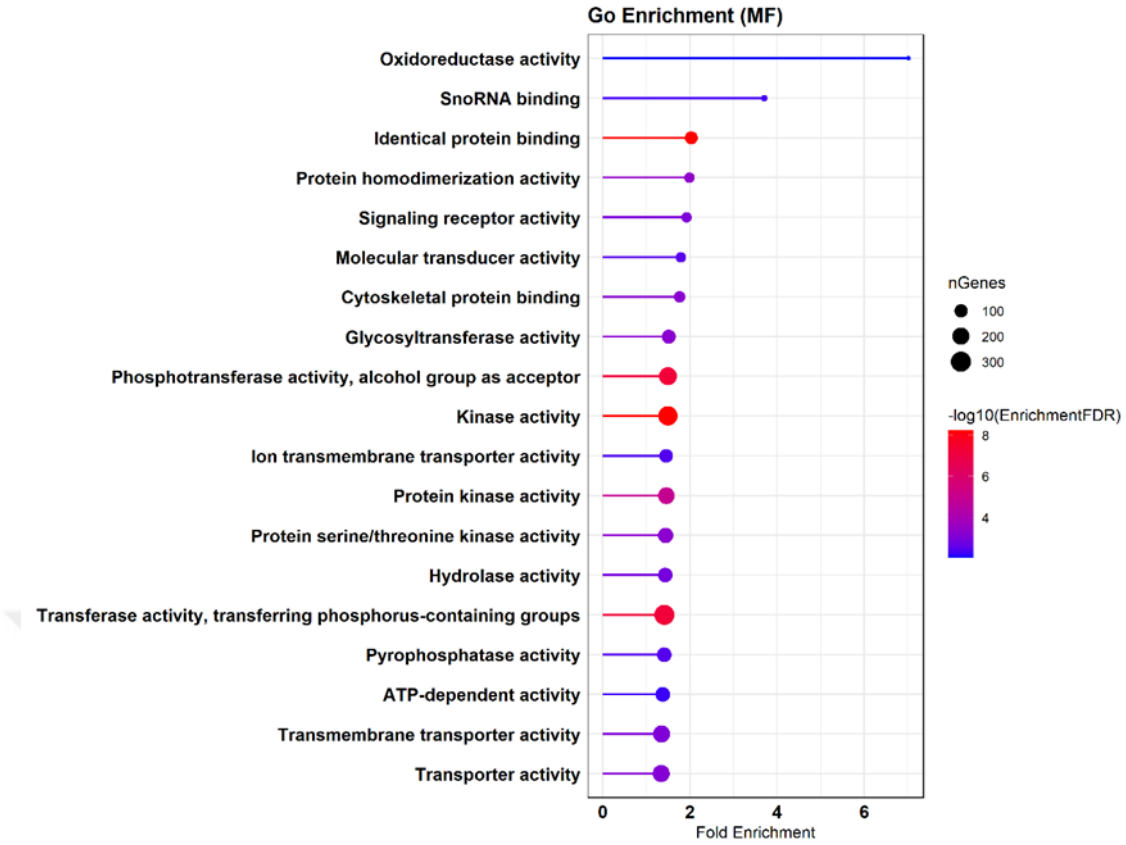


Figure 4.11. Gene ontology molecular function between '01-1N-58' genotype and 'Sarilop' cultivar

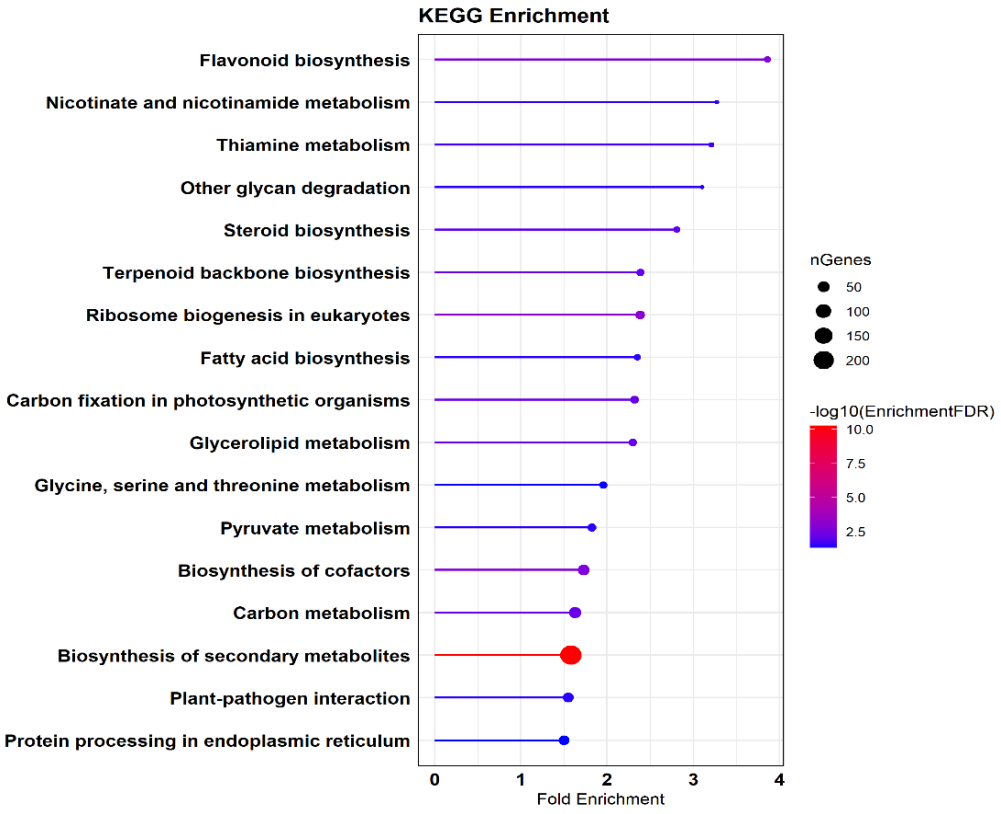


Figure 4.12. KEGG pathway analysis between '01-1N-58' genotype and 'Sarilop' cultivar

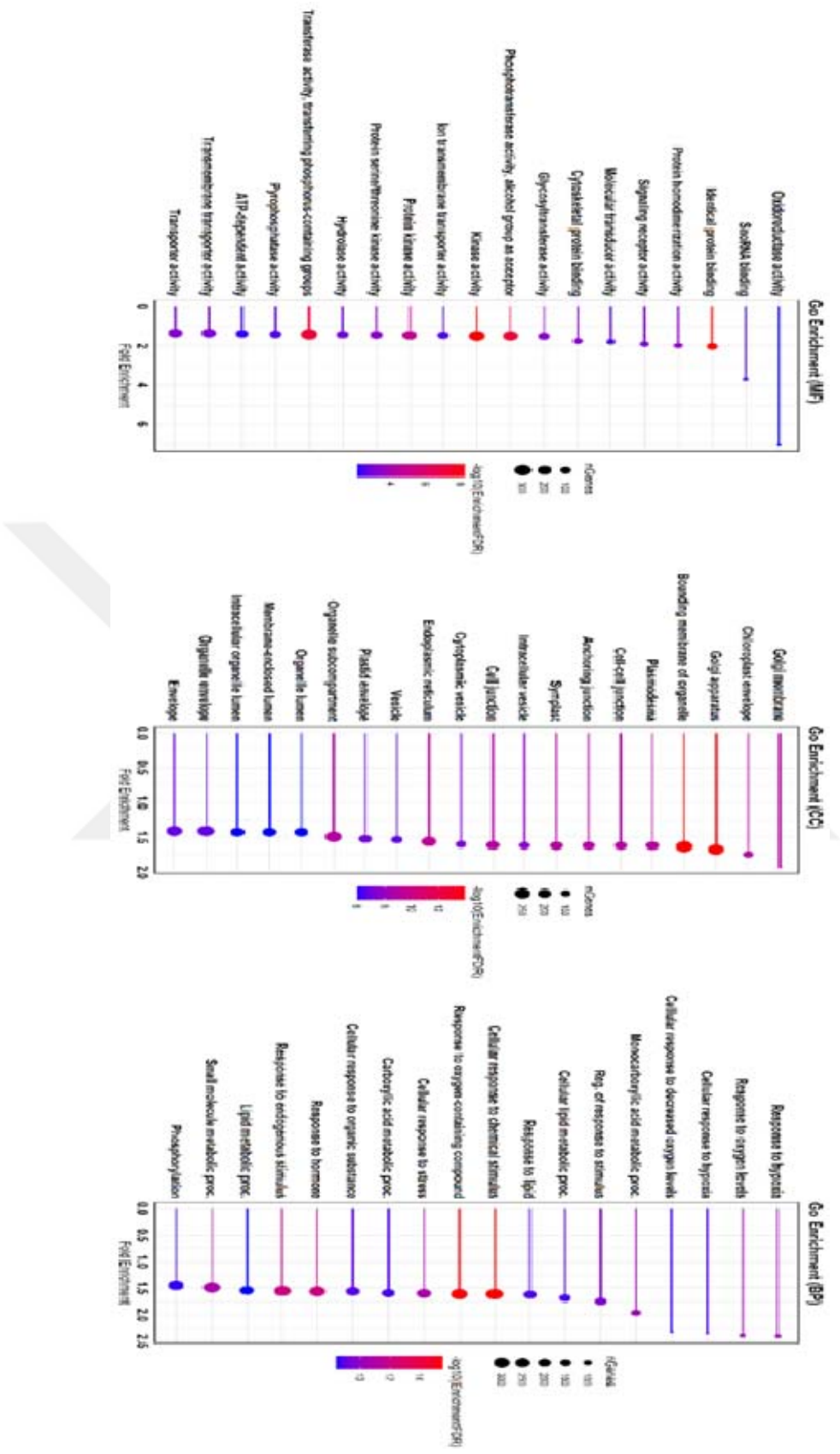


Figure 4.13. Gene ontology MF, CC and BP between ‘01-1N-58’ genotype and ‘Sarilop’ cultivar

When the biosynthesis of secondary metabolites pathway is examined, it is located in the green circle frame (Figure 4.14).

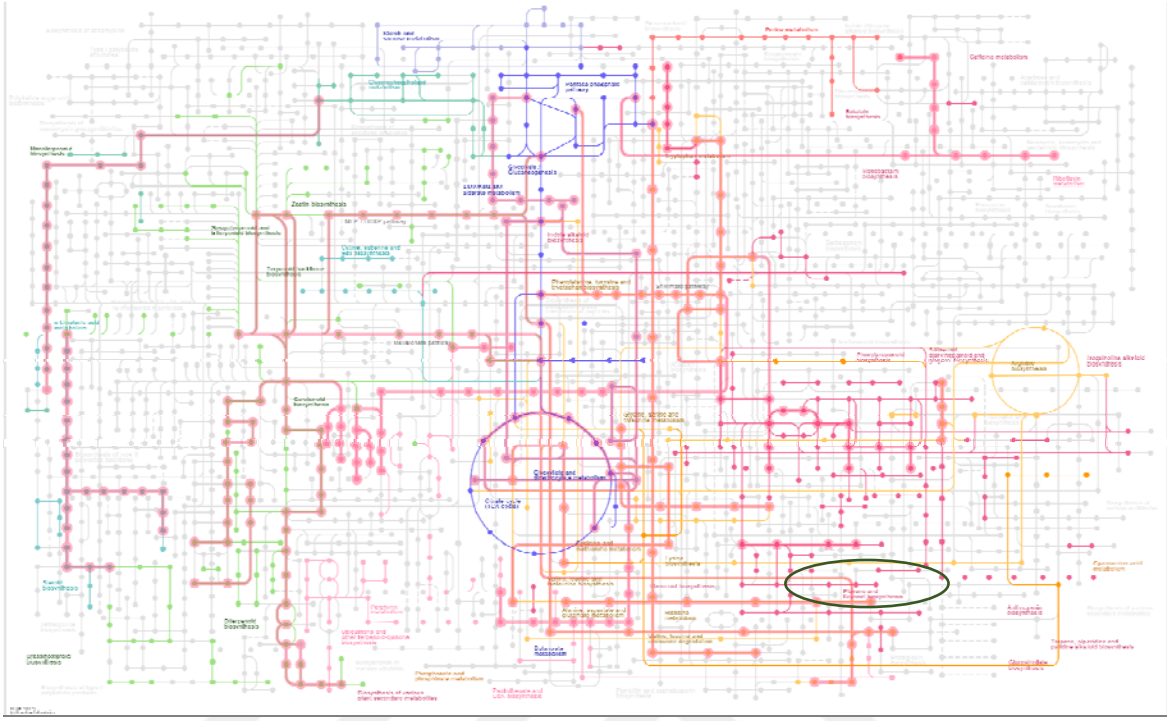


Figure 4.14. Biosynthesis of secondary metabolites pathway

4.7. Results of cDNA synthesis and primer optimization

This research was carried out in the molecular laboratory of the Horticulture Department at Çukurova University in Adana. The "01-1N-58" genotype and "Sarilop" cultivar were selected with their peels on the basis of their high and low anthocyanin contents based on HPLC-MS/MS results. Furthermore, the qRT-PCR technique was used to examine the expression levels of four different genes and one endogenous control involved in anthocyanin synthesis.

Control of synthesized cDNA libraries as endogenous control was carried out with the primer of the *PgRPSII* gene. The cDNA libraries and PCR reactions were performed using the *PgRPSII* primer. For PCR products, a 2% concentration was prepared, and later they were run on an agarose gel. After electrophoresis, the gel was visualized under UV light. After confirmation of cDNA synthesis for gene expression, the primers to be used were optimized. With the classical PCR reaction, the melting temperatures (Tm) of the primers were determined by bonding temperatures. The *PAL* Tm was optimized at (56 °C), *CHS* (54 °C), *CHI* (56 °C), *F3'H* (56 °C), and *PgRPSII* (54 °C).

4.8. qRT-PCR gene expressional profiles of genotypes

Although there are some expression studies carried out with genes associated with the anthocyanin biosynthesis pathway in many plants, including fruits. However, anthocyanin biosynthesis in *Ficus carica* L. related gene expression studies are limited. Anthocyanins are glucosides, which are flavonoid derivatives of the phenylpropanoid pathway. Therefore, fruits containing anthocyanins are not only aesthetically pleasing but also considered an attractive attribute of the fruit's quality. The high concentrations of phenolic compounds, especially anthocyanins, in *Ficus carica* L. are largely responsible for the plant's beneficial effects on health. Therefore, in recent years, molecular studies related to the anthocyanin biosynthesis pathway have gained importance. Beside this importance, however, fig molecular studies on the anthocyanin biosynthesis mechanism are limited.

Based on the HPLC/MS-MS results, the "01-1N-58" (dark-purple) genotype depicted the high anthocyanin content in their peels in the experiment. On the contrary, the Sarilop (greenish-yellow) cultivar exhibited the least anthocyanin content in its peels. qRT-PCR was used to validate the RNA-seq gene expression level results. The 2–ΔΔCt formula was used to calculate gene expression levels, and the results are shown in (Figures 4.15, 4.16, 4.17, and 4.18).

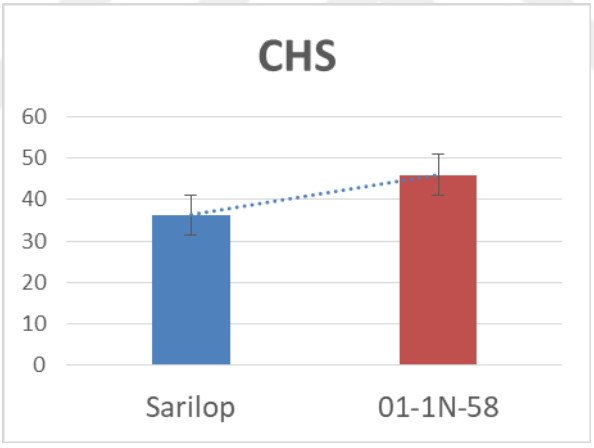


Figure 4.15. Expression levels of the *CHS* gene in the peels of Sarilop and 01-1N-58.

As a result of the gene expression analysis, it was observed that the *CHS* gene in '01-1N-58' (dark-purple) fig genotype showed relatively high expression over 'Sarilop' (greenish-yellow) cultivar. In 01-1N-58 genotype the expression level was noted as 45 whereas, in Sarilop the expression level was recorded as 35. In various studies the high level of *CHS* was also found on the peels of dark cultivars of the figs as compared to the yellow figs (Wang et al., 2017; Wang et al., 2019; Lama et al., 2020; Li et al., 2020; Li et al., 2021; Unal et al., 2021).

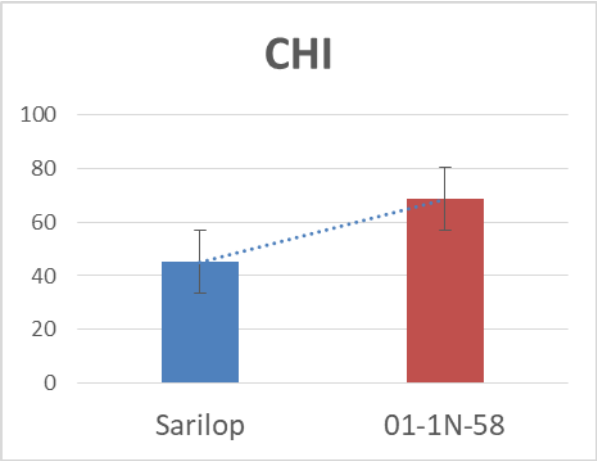


Figure 4.16. Expression levels of the *CHI* gene in the peels of Sarilop and 01-1N-58.

A vast variety of natural compounds are biosynthesized by the enzymes of the CHS-superfamily in plants. They are crucial for the pigmentation of flowers, UV protection, and phytopathogen defense. As a result, the CHS-superfamily provides a useful model for studying the rates and patterns of sequence variation among duplicate genes. Several copies of the CHS genes were discovered in several plants (Yang and Gu, 2006). The gene expression analysis depicted that the *CHI* gene in '01-1N-58' fig genotype showed relatively high expression over 'Sarilop' cultivar. In 01-1N-58 genotype, the expression level was noted as 70 whereas, in Sarilop the expression level was recorded as 50. Both fig genotypes showed higher level of *CHI* expression over *CHS*. In various studies the high level of *CHI* was also found on the peels of dark cultivars of the figs as compared to the yellow figs (Wang et al., 2017; Wang et al., 2019; Lama et al., 2020; Li et al., 2020; Li et al., 2021 Unal et al., 2021).

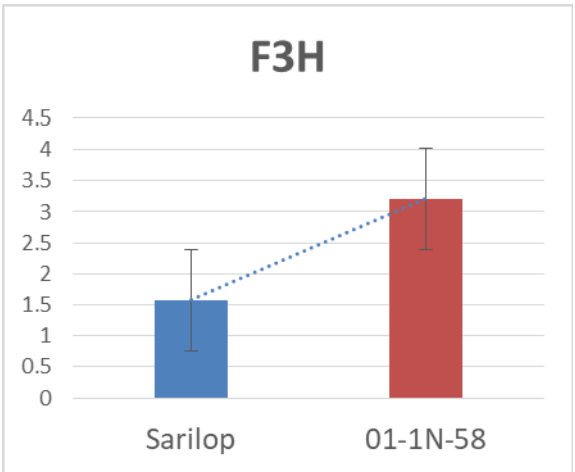


Figure 4.17. Expression levels of the *F3H* gene in the peels of Sarilop and 01-1N-58.

The gene expression analysis depicted that the *F3H* gene in '01-1N-58' fig genotype showed relatively high expression over 'Sarilop' cultivar. In 01-1n-58 genotype, the expression level was noted as 3.2 whereas, in Sarilop the expression level was recorded as 1.5. Both fig genotypes showed a less level of overall *F3H* gene expression over *CHI* and *CHS*. In various studies the high level of *F3'H* was also found on the peels of dark cultivars of the figs as compared to the yellow figs (Wang et al., 2017; Wang et al., 2019; Lama et al., 2020; Li et al., 2020; Li et al., 2021; Unal et al., 2021).

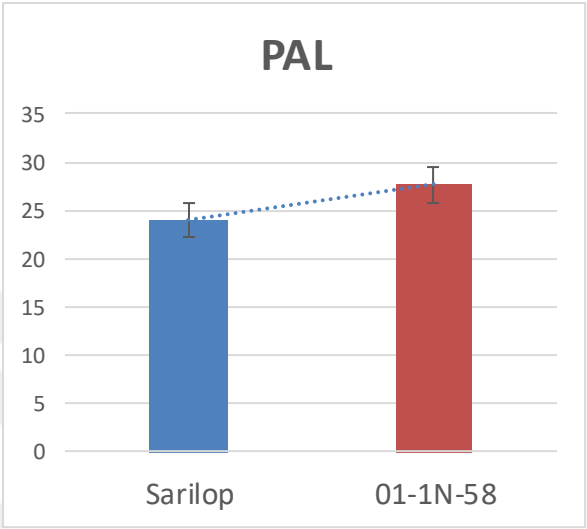


Figure 4.18. Expression levels of the *PAL* gene in the peels of Sarilop and 01-1N-58.

The gene expression analysis revealed that the *PAL* gene in the '01-1N-58' fig genotype demonstrated the relatively high expression compared to the 'Sarilop' cultivar. The expression level was observed as 27 in the 01-1n-58 genotype and 23 in the Sarilop genotype. Overall, both the cultivar and genotype depicted a less level of *PAL* gene expression level as compare to *CHI* and *CHS*. However, the expression level of *PAL* was higher than the gene *F3H*. In various studies the high level of *PAL* was also found on the peels of dark cultivars of the figs as compared to the yellow figs (Wang et al., 2017; Wang et al., 2019; Lama et al., 2020; Li et al., 2020; Li et al., 2021; Unal et al., 2021).

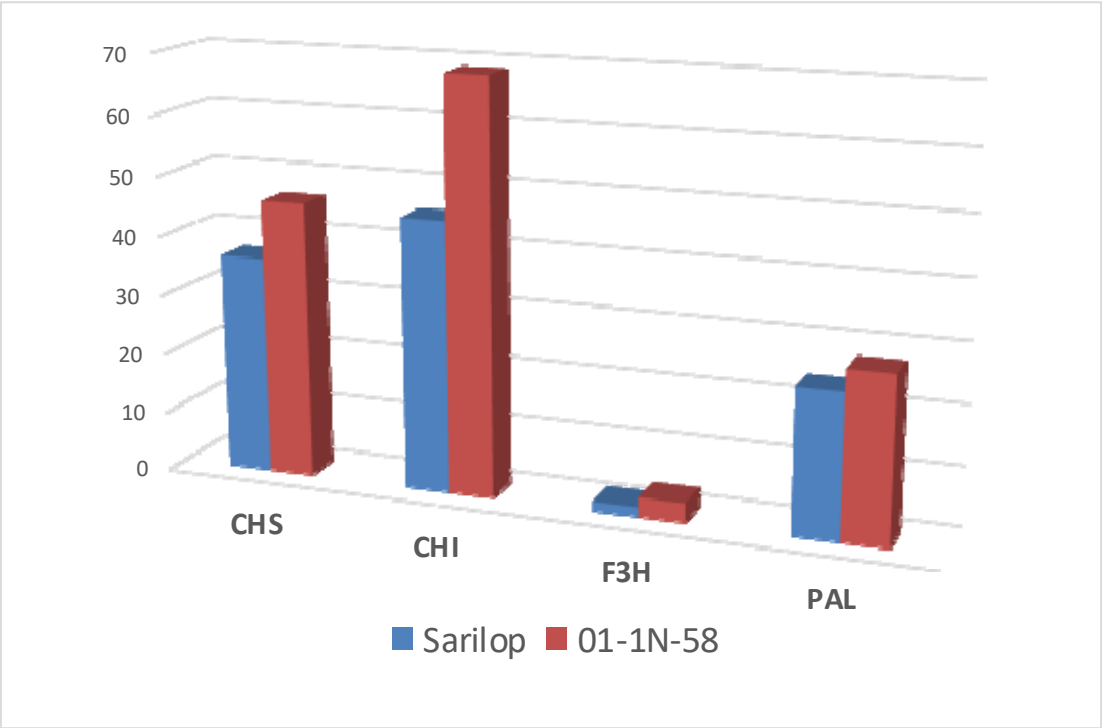


Figure 4.19. Expression levels of the four genes in the peels of Sarilop and 01-1N-58.

Expression-level analysis for anthocyanin was carried out on the peels of two Turkish figs. "01-1N-58" (dark-purple) genotype revealed the highest anthocyanin content in their peels, while the Sarilop (greenish-yellow) cultivar exhibited the least anthocyanin content in its peels. In quantitative real-time PCR studies, four different genes associated with anthocyanin biosynthesis were used: *PAL*, *CHS*, *CHI*, and *F3'H. PgRPSII* was used as an endogenous control gene. Overall, the results indicated that the CHI gene showed a high expression level in both genotypes. However, the dark color genotype "01-1N-58" exhibited the highest expression of *CHI* over the greenish-yellow Sarilop cultivar. Moreover, the second highest expression level was noted as *CHS* in both genotypes.

The *PAL* gene was expressed as the third highest, whereas the least expression was observed on the *F3H* gene on both the genotype and cultivar. More importantly, the dark-purple peels of the 01-1N-58 genotype with high anthocyanin exhibited the highest level of expression of all four genes. On the contrary, the peels of the greenish-yellow Sarilop cultivar with low anthocyanin depicted the least level of expression of all four genes. These qRT-PCR analyses validated the RNA-seq expression results in our experimental fig genotype and cultivar.

Likewise, a study conducted by Li et al. (2020) on the fruit peels of figs compared the two stages the red and yellow stages. After transcriptomic analysis, they obtained five *CHS* and three *CHI* genes, along with other important genes as well. They conducted qRT-PCR and validated the results of RNA-seq. All the genes, including *CHS*, *CHI*, *PAL*, F3'H, *C4H, DFR, ANS* and *UFGT,* showed high gene expression levels in the red fig genotype. However, like our results, all the above-mentioned genes showed the lowest expression level in the yellow stage fig genotype. Other qRT-PCR studies also validated our results and confirmed the high expression of *CHS, CHI, PAL* and *F3'H* genes in the dark peels of fig cultivars (Wang et al., 2017; Wang et al., 2019; Lama et al., 2020; Li et al., 2021; Unal et al., 2021).

5. CONCLUSION AND RECOMMENDATIONS

As per our knowledge, this research presents the most extensive analysis of total phenolics, sugars and antioxidant capacity in both the peels and pulps of eight Turkish figs (*Ficus carica* L.). In this study, substantial phytochemical variations in the fig cultivars and genotypes were observed during a tree ripening stage. Detection and characterization analysis of phytochemical compounds in key cultivars and genotypes will also ensure the roadmap for high-quality fig fruit production in Turkiye. HPLC-MS-MS phenolic analysis method was performed and detected 31 significant phenolic compounds (7 colored anthocyanins and 24 colorless PCs). The predominant anthocyanin identified was cyanidin 3-O-rutinoside, whereas chlorogenic acid was determined to be a key colorless phenolic compound in the experimented samples. In our eight experimented fig cultivars and genotypes, the genotype '01-1N-58' displayed the highest total PCs and hence a higher antioxidant activity detected in both their peels and pulps than the other Turkish experimented fig cultivars and genotypes. Of the total sugar contents, glucose was the most abundantly found sugar in the skin and fructose in pulps.

Turkiye is the most important country in terms of the production of figs. 'Sarilop' and 'Bursa Siyahi' are the most commonly grown fig cultivars in Turkiye. However, in our study, the 'Sarilop' cultivar had the least PCs and antioxidant activity detected among all the experimented samples. In this study, PCA analysis was also performed, which validated the significant differences in chemical composition between cultivars and genotypes of fig fruit. It also confirmed that some common characteristics of genotypes, particularly '01-1N-58', were found to have a higher level of polyphenols.

Additionally, this study utilized RNA-Seq analysis to compare gene expression in two different fig varieties, the dark-purple '01-1N-58' genotype and the greenish-yellowish 'Sarilop' cultivar. A large number of genes were identified, with the '01-1N-58' genotype having 39,430,05 unigenes and 38,325,911 duplicate genes, while the 'Sarilop' cultivar had 43,348 unique genes and 44,608,828 duplicate genes. Differential gene expression analysis revealed 21102 differentially expressed genes between the two samples, with 3542 genes up-regulated and 3164 genes down-regulated in the '01-1N-58' genotype. The results of this study provide valuable insights into the genetic differences between fig varieties, which could be useful for further studies in fig breeding and production. Furthermore, qRT-PCR analysis was also conducted to validate the results of the expression of genes in RNA-seq. Four genes *CHI*, *CHS*, *PAL* and *F3'H* showed higher expression levels in the peels of the dark-purple O1-1N-58 genotype and depicted lower expression levels in the greenish-yellow Sarilop cultivar.

Therefore, based on the results, the genotype '01-1N-58' may be conserved as a breeding material in the future for fig producers and consumers because they are ready-to-use materials and could also be handy to promote human health as they are rich in nutritional

supplements, natural antioxidants and phenolics. This study has important nutritional information about the content of sugars in the peels and pulps of fresh figs.

Additionally, the study found that the peels of figs, particularly the dark peels had more prominent levels of phenolics and antioxidant capacity than the yellow peels and pulps of all experimented fig cultivars and genotypes. According to the findings of the current study, it is recommended to consume fig fruits as a whole with their dark peels. Moreover, significant differences in biochemical and antioxidant potential across cultivars and genotypes suggest that they have enriched phytochemical profiles that demonstrate a notable approach to converting fig genotypes into value-added food ingredients.

The outcomes of this study are not only a fruitful interest to assist our understanding of the constitution and biological functions of various fig peels and pulps, but also have a critical impact on nutrition and human health as well. However, more research is required to investigate the biological activities and genomic studies including transcriptomics to support their beneficial potential in the human diet as well as to explore the key genes specifically responsible for the synthesis of key phenolic compounds such as anthocyanins in dark *Ficus carica* L. peels.

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