T.C
ÇANAKKALE ONSEKIZ MART UNIVERSITY
SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOMOLECULAR SCIENCES

CHARACTERIZATION OF STEROL DELTA 7 REDUCTASE GENE INVOLVED IN PHYTOSTEROL BIOSYNTHESIS OF OLIVE

MASTER OF THESIS

REŞAT EŞİYOK

THESIS ADVISOR:
Prof. Dr. Kemal Melih TAŞKIN

ÇANAKKALE – 2023

T.C
ÇANAKKALE ONSEKIZ MART UNIVERSITY
SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOMOLECULAR SCIENCES

CHARACTERIZATION OF STEROL DELTA 7 REDUCTASE GENE INVOLVED IN PHYTOSTEROL BIOSYNTHESIS OF OLIVE

MASTER OF THESİS

REŞAT EŞİYOK

THESIS ADVISOR:
Prof. Dr. Kemal Melih TAŞKIN

This study has been supported by The Scientific and Technological Research Council of Türkiye

Project No: 118O405

ÇANAKKALE – 2023

T.C.

ÇANAKKALE ONSEKIZ MART UNIVERSITY

SCHOOL OF GRADUATE STUDIES

The dissertation entitled "**Characterization of Sterol Delta 7 Reductase Gene Involved in Phytosterol Biosynthesis of Olive**", was submitted by **Reşat EŞİYOK** under the supervision of Prof. Dr. Kemal Melih was defended on **30.01.2023**. The Examining Committee Members have approved this dissertation as **Master of Science in Biomolecular Sciences** of Çanakkale Onsekiz Mart University School of Graduate Studies.

.

EXAMİNİNG COMİTTEE	**Signature**
Prof. Dr. Kemal Melih TAŞKIN Supervisor	……
Asst.Prof. Fatih SEZER	……
Asst.Prof. Kaan HÜRKAN	…….

Thesis Number : ……..

Thesis Defense History : 30/01/2023

Assoc. Prof. Dr. Yener PAZARCIK

Director School of Graduate Studies

20/02/2023

PLAGIARISM DECLARATION PAGE

I declare that all the information and results presented in visual, auditory, and written form were obtained by myself in accordance with academic and ethical criteria and that all other results and material referenced in the thesis but not pertinent to this study investigation are cited.

ETİK BEYAN

Çanakkale Onsekiz Mart Üniversitesi Lisansüstü Eğitim Enstitüsü Tez Yazım Kuralları'na uygun olarak hazırladığım bu tez çalışmasında; tez içinde sunduğum verileri, bilgileri ve dokümanları akademik ve etik kurallar çerçevesinde elde ettiğimi, tüm bilgi, belge, değerlendirme ve sonuçları bilimsel etik ve ahlak kurallarına uygun olarak sunduğumu, tez çalışmasında yararlandığım eserlerin tümüne uygun atıfta bulunarak kaynak gösterdiğimi, kullanılan verilerde herhangi bir değişiklik yapmadığımı, bu tezde sunduğum çalışmanın özgün olduğunu, bildirir, aksi bir durumda aleyhime doğabilecek tüm hak kayıplarını kabullendiğimi taahhüt ve beyan ederim.

Reşat EŞİYOK

20.02.2023

ACKNOWLEDGEMENT

Life has strange ways of teaching us the importance of challenging work, adaptability, support group, persistence, compassion, and courage. Many people have played important roles in the achievement of this induvial and yet collective milestone.

I would like to thank several people beginning with my mentor and advisor, Prof. Dr. Kemal Melih TAŞKIN. Thank you for having faith in me. I lost all faith at times, but my advisor always encouraged me and was a significant help to me. Your operational guidance has been my compass in the winding adventure as I completed my master's.

I will be eternally grateful to the graduate committee for their willingness to assist me throughout this process. Finally, this success would not have been possible without the assistance of Dr. Fatih SEZER and Dr. Aslıhan ÖZBİLEN. This accomplishment would not have been possible without your encouragement and assistance during challenging times.

Finally, Dr. Deniz Çelik, you have been both my teacher and my brother. Sir, you are more than a teacher to me. Thank you, a lot, for everything. It was an immense pleasure to meet you.

I am grateful to my father, Mehmet EŞİYOK, and my mother, Besra EŞİYOK, for their unwavering trust, timely encouragement, and unending patience. When I was tired, it was their love that helped me get back up. Despite the long distance between us, my sisters, and brothers, as well as my nephews, Azra, Ronya, and Azad, have been generous with their love and encouragement.

For their financial supports, I would like to thank The Scientific and Technological Research Council of Turkey (Project No: 118O405).

Reşat EŞİYOK

Çanakkale, January 2023

ÖZET

ZEYTİN'DE (*OLEA EUROPAEA* L.) FİTOSTEROL BİYOSENTEZİNE KATILAN STEROL DELTA 7 REDUKTAZ GENİNİN TANIMLANMASI

Reşat EŞİYOK

Çanakkale Onsekiz Mart Üniversitesi

Lisansüstü Eğitim Enstitüsü

Biyomoleküler Bilim Anabilim Dalı Yüksek Lisans Tezi

Danışman: Prof. Dr. Kemal Melih TAŞKIN

Zeytin (*Olea Europaea* L.) bitkisi ülkemiz ve dünya için önemli bir tarım ürünüdür. Akdeniz bölgesinden başlayarak Ege, Marmara ve Güney Anadolu Bölgesinde yetiştirilen bir bitkidir. Zeytin yağının içerisinde bulunan vitaminler, antioksidan ve diğer maddelerden dolayı insan sağlında kozmetik ve tıbbı alanda kullanılan önemli bir üründür. Zeytin yağında bitkisel yağ olarak adlandırılan fitosteroller bulunmaktadır. İçermiş olduğu vitaminler ve diğer maddelerden dolayı kalp damar hastalıklarına, kolesterol ve Alzheimer gibi çeşitli hastalıklara karşı kullanıldığında olumlu yönde etkileri olduğu bilimsel araştırmalar ile ortaya çıkarılmıştır. Bu çalışmada, fitosterol biyosentezinden görev alan önemli bir görevi olan Sterol Delta 7 redüktaz (*OeDWF5)* genin sekiz farklı evresinde toplanılan zeytin meyvelerindeki gen anlatımı Real-Time PCR ile incelenmiştir. Çalışmada, *OeDWF5* genin en fazla beşinci evresindeki Gemlik çeşidinde ve Memecik çeşidin de ifade edildiği ortaya çıkmıştır. *OeDWF5* geni altıncı evrede ise sırasıyla Ayvalık, Gökçeada, Memecik ve Gemlik çeşitlerinde ifade edilmiştir. Diğer dönemlerden elde edilen verilere göre, gen anlatımları istatiksel olarak önemli bir fark bulunmamıştır.

Anahtar Kelime: Fitosterol, *Olea Europaea* L.*, OeDWF5,* Real-Time PCR

ABSTRACT

CHARACTERIZATION OF STEROL DELTA 7 REDUCTASE GENE INVOLVED IN PHYTOSTEROL BIOSYNTHESIS OF OLIVE

Reşat EŞİYOK

Çanakkale Onsekiz Mart University

School of Graduate Studies

Master of Sciences Thesis in Biomolecular Sciences

Advisor: Prof. Dr. Kemal Melih TAŞKIN

Olive (*Olea europaea* L.) is an important agricultural product for Türkiye and the world. Olive is a plant native to the Mediterranean regions that are grown in the Aegean, Marmara, and Southern Anatolia regions in Türkiye. Since olive oil contains vitamins, antioxidants, and other substances, olive is a vital plant used in cosmetics and medicine for human health. The phytosterol are one of the important components of vegetable oil. Because of the vitamins and other substances, olive oil contains, some scientific research has shown that it has positive effects when used against various diseases such as cardiovascular disease, cholesterol, and Alzheimer's. In this study, gene expression of the Sterol Delta 7 Reductase (*OeDWF5)* gene, an essential gene involved in phytosterol biosynthesis, was examined using Real-Time PCR from data obtained from olive fruits collected at eight different ripening stages. According to the results, a high level of expression of the *OeDWF5* gene was in Gemlik and then Memecik cultivars among the cultivars in the fifth period. According to the data obtained in all cultivars, the expression level of the *OeDWF5* gene was high in the Ayvalık, Gökçeada, Memecik and Gemlik varieties, respectively. There was no significant statistical difference in gene expression based on information at later ripening periods.

Keywords: phytosterol, *Olea europaea* L. *OeDWF5,* Real-Time PCR

TABLE OF CONTENTS

Page No

ABBREVIAOTINS AND ICONS

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
cDNA	Complementary DNA
RT-PCR	Real-Time Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
PCR	Polymerase Chain Reaction
OeDWF5	Sterol Delta 7 Reductase
DWF7	Delta 7- Sterol-C5-Desaturase
SMT1	Sterol Methyltransferase 1
SMT2	Sterol Methyltransferase 2
SDR	3-Oxosteroid Reductase
Bp	Base Pair
Mg	Milligram
Ng	Nanogram
µl	Microlite
DNase	Deoxyribonuclease
mRNA	Messenger Ribonucleic Acid
DEPC	Diethyl Pyro carbonate
EPTA	Ethylenediamine Tetra acetic Acid
Ml	Millilitre
RNase	Ribonuclease
FAO	Food and Agriculture Organization

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Olive, or *Olea europaea L.*, is acknowledged as one of the world's oldest cultivated and major profitable plants (Green, 2002). With a high rate of morphological and biological variability (S.M. Jain et al.,2005), and allogamous "wild" (*Olea europaea* var. *sylvestris*) and "cultivated" species (*Olea europaea* L. subsp. *europaea* var *europaea*) (Azimi et al, 2015). Thanks to its strong adaptation to long and hot summers (Yamani et al, 2020). In 2021 (FAO), the highest olive oil production was recorded in Spain, Greece, Italy, and Türkiye, the major olive-producing countries. The stated counties have an overwhelming percentage (80%), with Türkiye producing 1738680 tons of the world's production olives in 2021. The olive has proved to be an unprecedented and indispensable part of Mediterranean geography (Anonymous, 2023) (Figure 1).

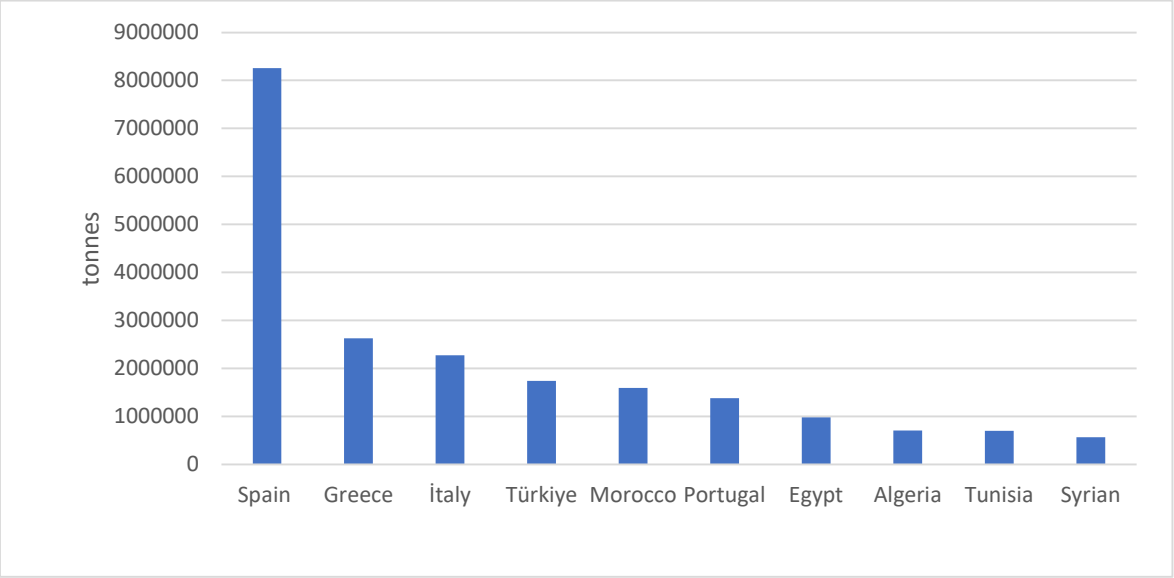


Figure 1. Olive production in the World in 2021 (Anonymous, 2023)

In this thesis, four different local olive varieties, including Memecik, Ayvalık, Gemlik, and Gökçeada were used. These varieties are drought resistant, which are important characteristic for cultivation (Saplings, 2012). Ayvalık is a well-known variety in the Marmara region. The Ayvalık is an excellent (Andjelkovic et al, 2009) and the most widely used variety in Türkiye (Varol et al, 2009) and (Tümen et al, 2013) because of tits rich oleic

acids and tocopherols contents. Memecik variety is known from the Aegean region and reportedly accounts for 45 % of the total olive trees in Türkiye. Ayvalık variety usually is growth in the Marmara and Aegean regions. This study reported that are used for olive oil and table olives (Kıralan and Bayrak, 2013). Memecik, for instance, is of vital variety because of its high-quality contents of oils, total sugar, protein, and total phenolic compounds. Gemlik cultivar, used as table olives and oil (Canözer, 1991), has the most abundant content of chemicals such as chlorophyll and carotenoid (Sevim et al, 2016). Gökçeada contains the lowest α-tocopherol and γ-tocopherol content compared to Gemlik, Ayvalık and Memecik varieties (Şeker et al, 2008). Finally, within the legitimate boundaries of the natural resistance and individual peculiarities, each variety proves specific data relating to the field in general, and Türkiye.

1.1. An Overview of The Phytosterol

The natural sterols synthesized in animals, plants, and fungi are also known as zoosterols, phytosterol, and mikosterols respectively (Kayahan M. 1998). The phytosterol synthesized from triterpenes are diverse structural components of plant membranes. Their main function is to stabilize the lipid bilayers (Hannich et al, 2011). Phytosterols, based on the number of methyl groups at the C-4 position, are structurally divided into the following three classes: 4-desmethyl sterols, 4-monomethyl sterols, and 4,4-dimethyl sterols. phytosterols are found in free or esterified states with fatty acids, phenolic acids, or glycosides (Quilez et al., 2003). In general, phytosterols and phytostanols (a fully saturated subgroup of phytosterol) are found in plant foods belonging to the 4-desmethyl sterols group. Commonly found phytosterol are campesterol, Stigmasterol, and especially Sitosterol. For the phytosterols, the common ones are sitostanol and campestanol (De Jong et al., 2003). The phytosterols are synthesized by mevalonate pathways (Schaller and Hubert, 2003).

Sterols are made up of a steroid skeleton with a hydroxyl group adjoined to the C-3 atom of the A-circle and an aliphatic flank chain annexed to the C-17 atom of the D-ring. The sterols molecule has a double bond, especially among C-5 and C-6 of the sterol component (Figure 2) (Cantrill and Kawamura., 2012).

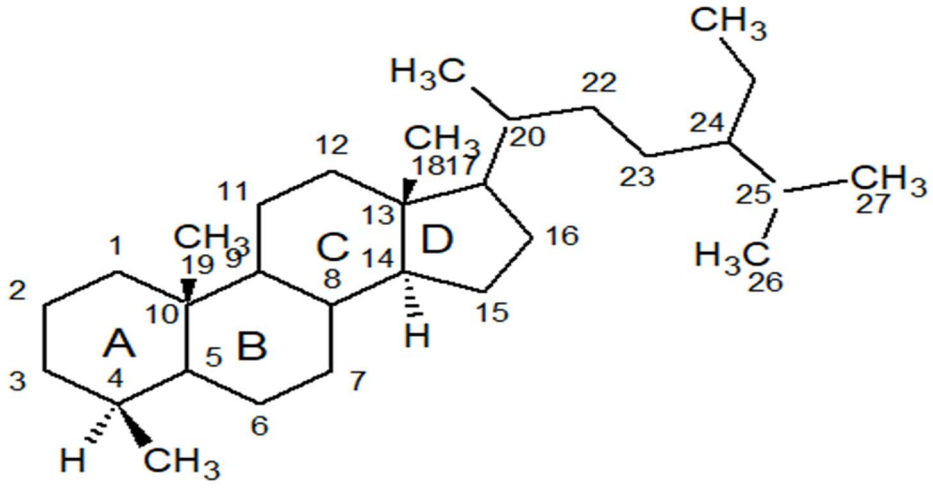
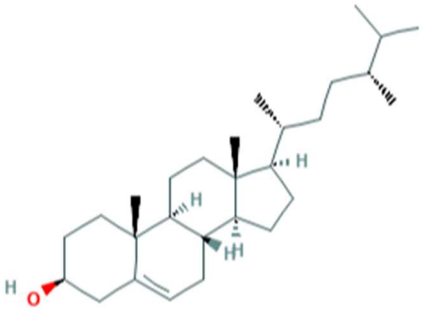
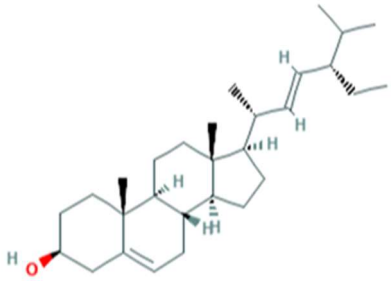


Figure **2.** General chemical formula of sterol (Anonymous, 2020)

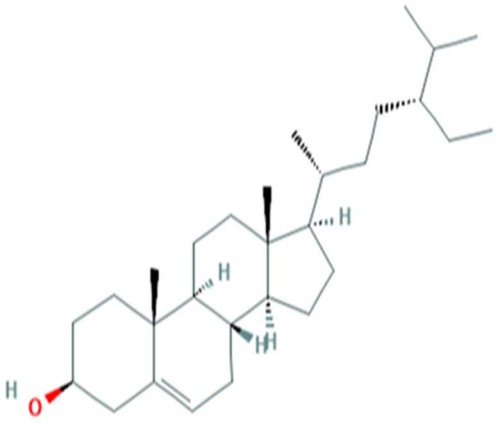
Several investigations have shown that there are over 250 different forms of sterols found in plant species. Also, phytosterol chemically resemble Cholesterol. (Figure 3) (Shahrad et al., 2017).

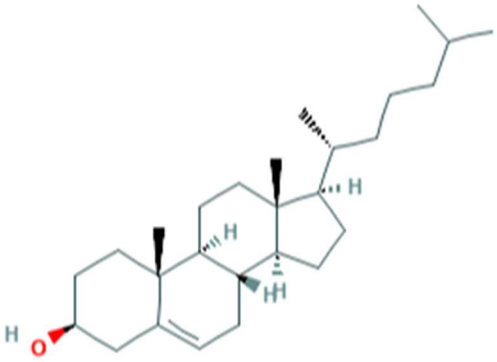


A) Campesterol



B) Stigmasterol





C) Sitosterol D) Cholesterol

Figure 3. Structural formulas of phytosterol and Cholesterol, A) Campesterol B) Stigmasterol C) Sitosterol D) Cholesterol. (Anonymous, 2021)

A recent study confirms the fact that phytosterol is present as a lipid component in eukaryotic organisms (Umate, 2016). Phytosterol is especially in plants' reproductive organs (Inês et al.,2019). Furthermore, phytosterol is thought to have a probable but significant role during evolution, and it is likely that it regulates the signaling process among molecules (Hannich et al, 2011). It means that it functions as a membrane dynamics regulator in membrane-bound protein modulation (Dufourm, 2008).

Phytosterol has a constructive function in higher plants for cell viability, embryogenesis, model development, cell division, chloroplasts, and the regulation of membrane-bound protein production and distribution such as enzymes and receptors (Lu et al, 2014). phytosterol enables the plant to adapt to drought conditions by regulating the plant metabolism (Kumar et al., 2015). They can also help plants to adapt to different environmental conditions and elevated temperatures (Jiwan et al, 1993). Moreover, structurally, in higher-up plants, animals, and insects, the phytosterol is the advanced guard of the steroid hormones which, on the other hand, provides for the developmental eukaryotic organisms (Carland et al, 2010) and (Benveniste, 2002). This research shows that the sterols are precursors of different functions molecular, and in particular, steroid hormones such as glucocorticoids, androgens, and estrogens in animals, ecdysteroids in insects, antheridia and

oogoniol in fungi, and BRs in plants (Clouse, 2002). Besides, sterols have increased the resistance to pathogens (Benveniste, 2002).

Phytosterol are important both for plants and humans. It is not synthesized in humans (Tarkowská and Danuše, 2019). It plays a crucial part in regulating human health because its chemical structure resembles Cholesterol (Shahrad et al., 2017). Therefore, phytosterols are important molecules against diseases. In a recent study, phytosterols have been shown a cholesterol-lowering effect in Caco-2 cells since they reduce the biosynthesis of Cholesterol, affecting key proteins of Cholesterol transport and metabolism, and interrupting the conversion of cholesterol to Cholesterol esters (Yuan et al, 2019). While phytosterol is a vital factor, Cholesterol has harmful effects on human health (Hannich et al, 2011). The foods rich in phytosterol esters are considered safe (Zhao et al, 2019).

Phytosterol is essential for a wide range of scientific and industrial processes. Secoiridoids, flavonoids, phenylpropanoids, phenolic alcohols, and phenolic acids are the most common phenolic families in olive fruits (Fernandez-Poyatos et al, 2019).

1.2. Phytosterol Biosynthesis

Phytosterol biosynthesis is vital for plants. The biosynthetic pathway for phytosterol starts with the (2E,6E) farnesyl diphosphate and, it is reacted with the enzyme farnesyl diphosphate farnesyltransferase to occur the squalene (Fu et al, 2019). This step catalyzed by three enzymes: Squalene Epoxidase 1, Squalene Epoxidase 2 and, Squalene Epoxidase 3 (Ploier et al, 2015). The squalene is converted to (3S)-2,3-epoxy-2,3-dihydrosqualene (Jaramillo-Madrid et al, 2019). In the next step, cycloartenol occurs when it is reacted with cycloartenol synthase (CAS 1) (Takemura et al, 2017). In the next reaction, 24-methylenecycartanol is occurred by sterol methyltransferase (SMT1) and sterol methyltransferase (SMT2) (Dennis and Nes, 2002) (Figure 4).

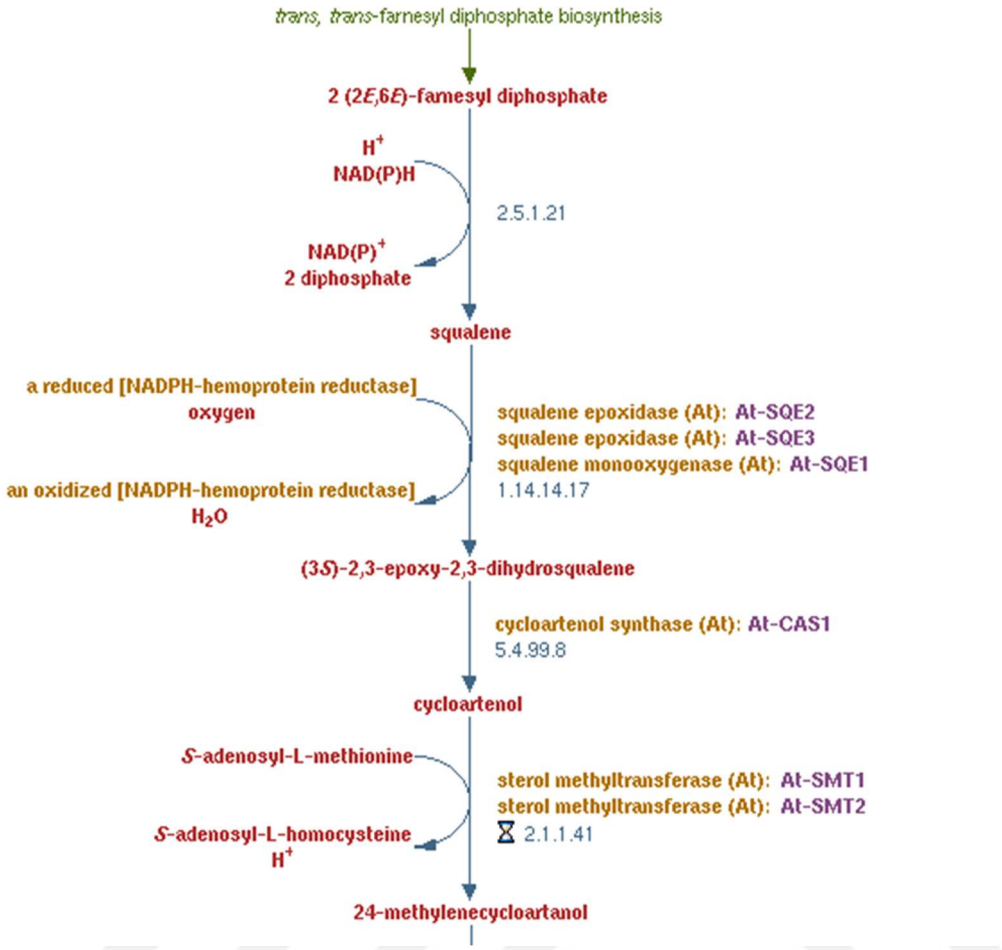


Figure 4. Synthesis of the 24-methylenecycloartanol (Anonymous, 2018)

Finally, 24-methylenecycartanol is converted to 4α-hydroxymethyl,4β,14α-dimethyl-9β,19-cyclo-5α- ergost-24(241)-en-3β-ol enzyme by 4-4-dimethyl-9β,19-cycloropylsterol-4α-methyl oxidase (Pascal et al, 1993). Phytosterol biosynthesis includes different chemical compounds, and it is a very long and complex mechanism because it has a lot of molecules. In these steps are synthesized 4α-formyl,4β,14α-dimethyl-9β,19-cyclo-5α-ergost-24(24^1)-en-3β-ol (Darnet and Rahier, 2004) (Figure 5).

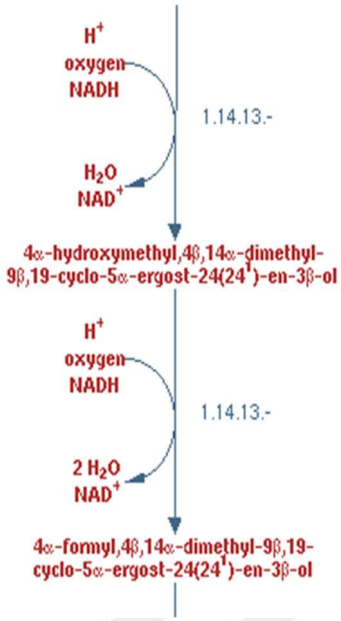


Figure 5. Synthesis of the 4α-formyl,4β,14α-dimethyl-9β,19-cyclo-5α-ergost-24(24^1)-en-3β-ol (Anonymous, 2018)

Here, it is interacted with NAD^+ and oxygen, and is consisted of 4α-carboxy-4β, 14α-dimethyl-9β,19-cyclo-5α-ergost-24(24^1)-en-3β-ol (Benveniste, 2002). At this stage, it, and NAD^+ has interacted with each other, and as a result, cycloeucalenone occurs. The reaction is catalyzed by the enzyme sterol-4-alpha-carboxylate 3-dehydrogenase (Rondet et al, 1999. The obtusifoliol is a vital compound role in the phytosterol biosynthesis. Finally, the cycloeucalenol has reacted. As a result, obtusifoliol has occurred and, here is used as the enzyme cycloeucalenol cycloisomerase (Benveniste, 2002)(Figure 6).

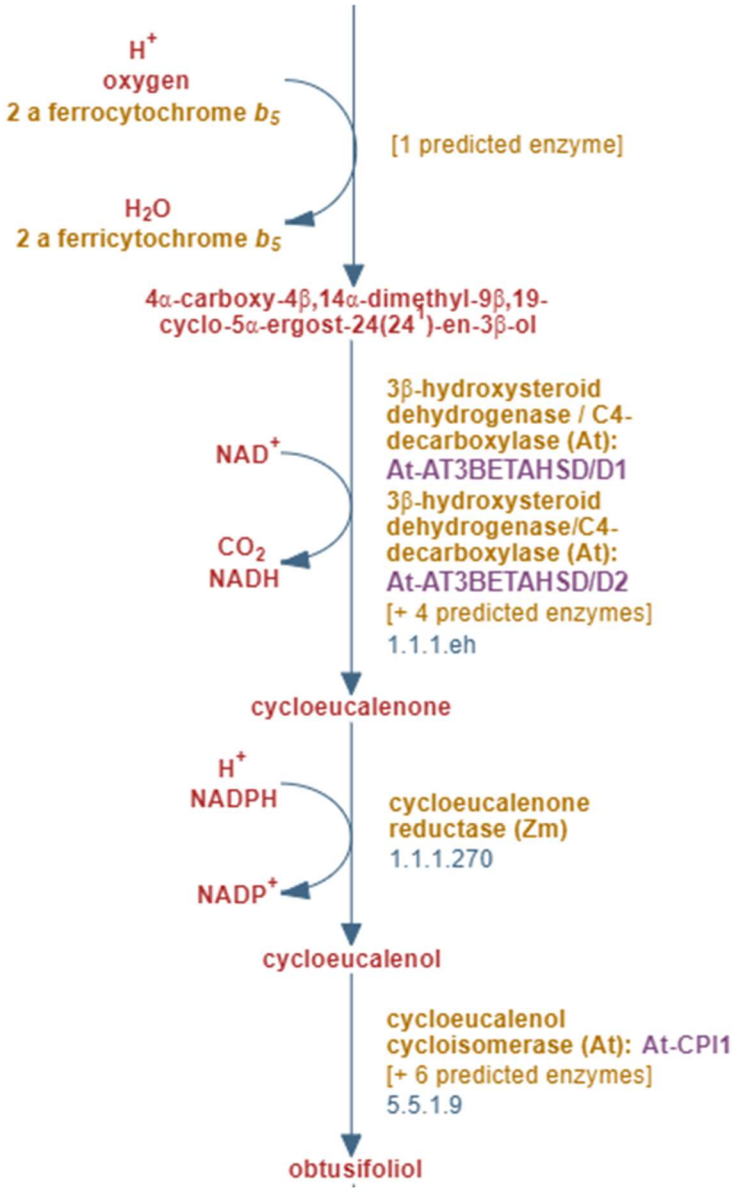


Figure 6. Synthesis of obtusifoliol (Anonymous, 2018)

In the following reaction, the obtusifoliol interacts with 3 reduced NADPH, 3 oxygen and, 2 H from the 4α-methyl-5α-ergosta-8,14,24(28)-trien-3β-ol. The enzyme the obtusifoliol-14α-demethylase catalyzes this reaction (Kushiro et al, 2001). In the next reaction, it interacts with the NADPH and, H+, consequently, convert to the 4α-methyl-5α-ergosta-8,24-dien-3β-ol after by catalyzing by the Δ14-sterol reductase enzyme (Kushiro et al, 2001). The C-8,7 sterol isomerase enzyme catalyzed the transformation of the 4α-methyl-5αergosta-8,24-dien-3β-ol into 24-methylenelophenol (Grebenok et al, 1998) (Figure 7).

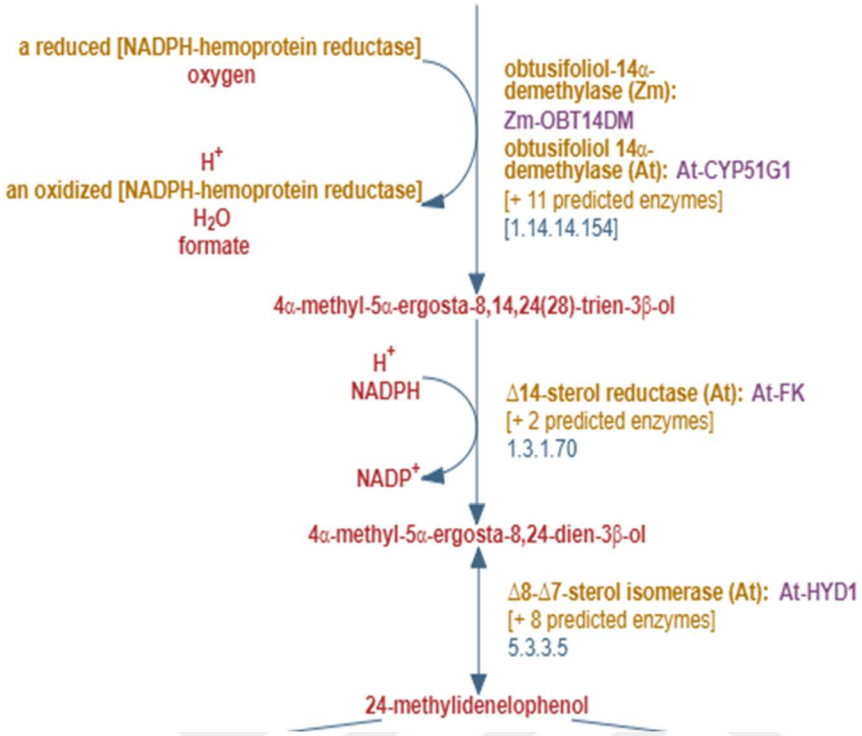


Figure 7. The synthesis of the 24-methylenelophenol (Anonymous, 2018)

Here, the phytosterol biosynthesis continues into two divisions. The first divisions, the Sitosterol, and Stigmasterol are synthesized, and the second branch, the Campesterol is synthesized (Choe et al,2000). The two branches are essential to the phytosterol biosynthesis. In the first branch, the 24-methylenelophenol is reacted, and 24-ethylidenelophenol occurs. (Choe et al,2000). Hence, it is catalyzed by sterol methyltransferase and sterol 24-C-methyltransferase. In their reaction, it is found as genes *SMT2, SMT3, SMT2-1,* and *SMT2-2,* respectively. The study shows that the *SMT2* is regulated by various hormones, and is provided to develop organs (Carland et al, 2002). In the following step, the 24-ethylidenelophenol reaches the reaction (Benveniste, 2002). As a result, 4α-hydroxymethyl-stigmasta-7,24(24^1)-dien-3β-ol is synthesized. In the following reaction, it is reacted reaction, and 4α-formyl- stigmasta-7,24(24^1)-dien-3β-ol is occurred (Benveniste, 2002) (Figure 8).

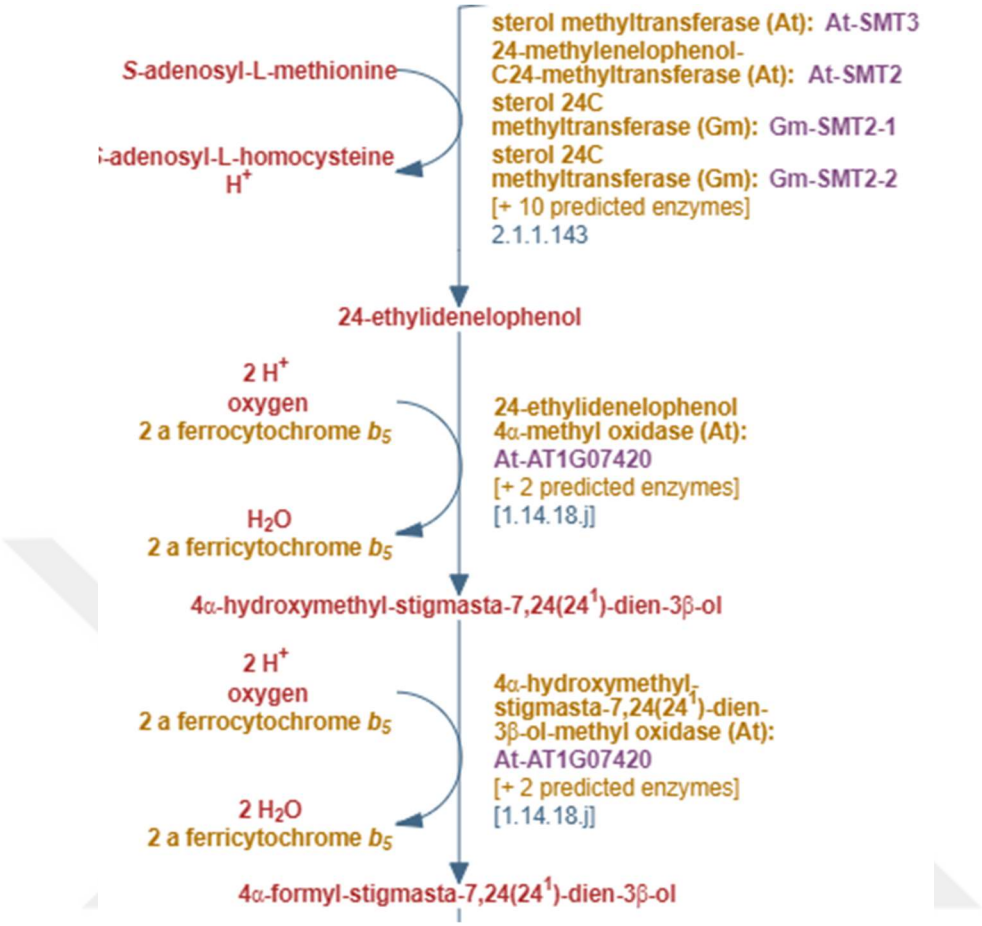


Figure 8. Synthesis of the 4α-formyl- stigmasta-7,24(24^1)-dien-3β-ol (Anonymous, 2018)

In the following step, it is reacted, and 4α-carboxy- stigmasta-7,24(24^1)-dien-3β-ol is converted, and the 4α-carboxy- stigmasta-7,24(24^1)-dien-3β-ol is interact with NAD^+ (Darnet and Rahier, 2006). At the end of the reaction occurs avenastenone (Benveniste, 2002). In the reaction that follows, the avenastenone is transformed into avenasterol. This reaction is catalyzed by the enzyme 3-oxosteroid reductase (SDR). In the next step, the avenasterol is reacted into the reaction, and then 5-dehydroavenasterol occurs. This enzyme is encoded by *DWF7* (Husselstein et al, 1999, Cho et al, 1999) (Figure 9).

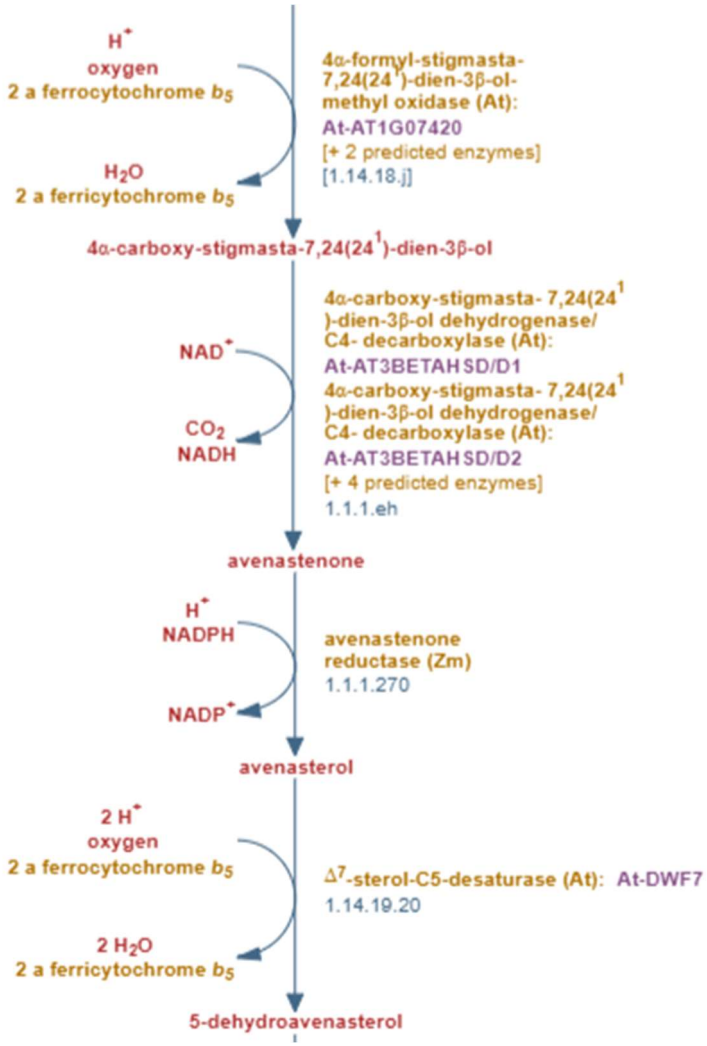


Figure 9**.** Synthesis of the 5-dehydroavenasterol (Anonymous, 2018)

In the reaction that follows, the 5-dehydroavenasterol is reacted to interact with the $NADPH^+$. At the end of the reaction, isofucosterol is produced. This reaction is predicted to be catalyzed by delta 7 reductase, and an enzyme is encoded by *OeDWF5* gene and, the transcript length for *OeDWF5* is 3719 bp. The *OeDWF5* activity is predicted for the endoplasmic reticulum membrane (Lecain et al, 1996). In the following step, the isofucosterol is reacted with the NADPH and H^+. After that, the delta 24-25- Sitosterol is formed. This reaction is catalyzed by isofucosterol isomerase (*DWF1*). The transcript length for the *DWF1* gene is 2699 bp. This investigation shows that the *DWF1* plays an essential part in phytosterol biosynthesis because it is an integral membrane protein which common likely is connected with the endoplasmic reticulum (Choe, Dilkes, et al, 1999).

In the following step, the delta 24-25- Sitosterol is reduced by NADPH and H^+. As a result, Sitosterol occurs. It is one of the most found among the phytosterol. The reaction is also catalyzed by the isofucosterol isomerase and encoded by the *DWF1* (Klahre et al, 1998) (Figure 10). The next reaction, Stigmasterol occurs by C-22 sterol-desaturase encoded by *CYP710A1* and *CYP710A2* genes. Additionally, the transcript length for the CYP710A1 is 1928 bp and the transcript length for the CYP710A2 is 1788 bp (Morikawa et al, 2006).

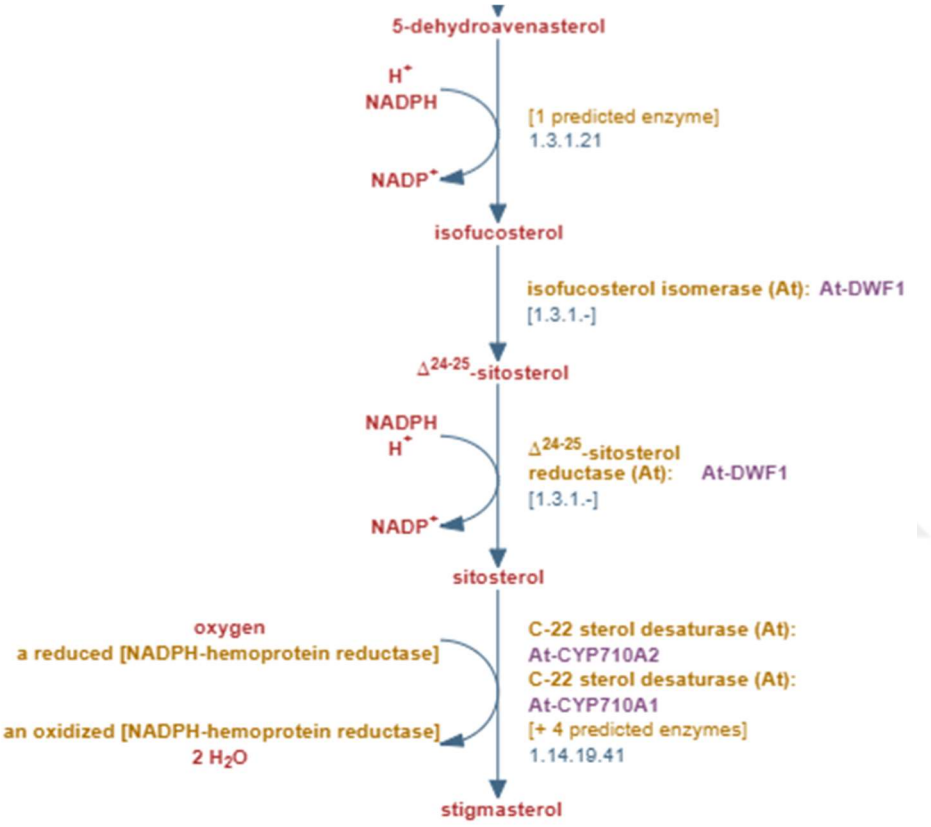


Figure 10. Synthesis of Stigmasterol (Anonymous, 2018)

In the second branch, the 24-methylenelophenol is transformed into 4α-hydroxymethyl-ergosta-7,24(24^1)-dien-3β-ol. In the following step, it is reacted, and 4α-formyl-ergosta-7,24(24^1)-dien-3β-ol occurs (Benveniste, 2002). In the next reaction, it is reacted. After that, 4α-carboxy-ergosta-7,24(24^1)-dien-3β-ol is occurred. Also, this reaction is predicted to be catalyzed by 4-α-methyl-delta7-sterol-4α-methyl oxidase. Additionally, this reaction is encoded by the gene AT1G07420 (Benveniste, 2002) (Figure 11).

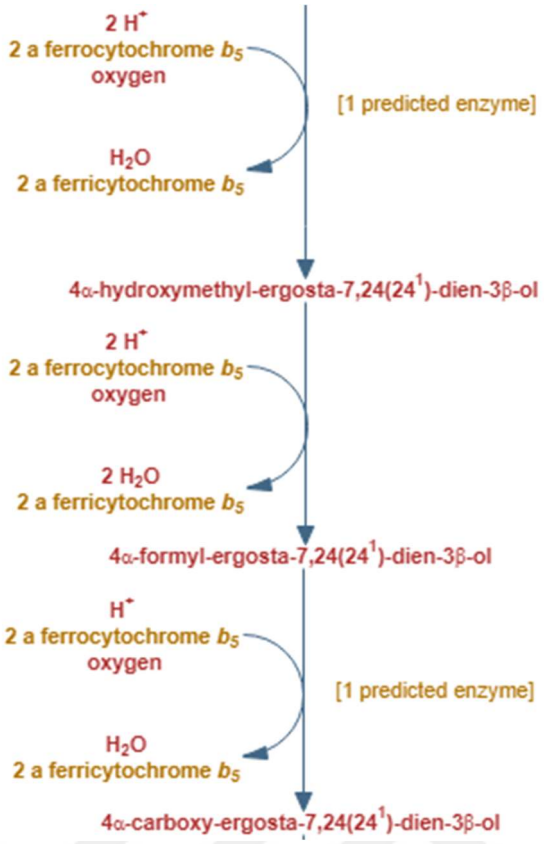


Figure 11. Synthesis of the 4α-carboxy-ergosta-7,24(24^1)-dien-3β-ol (Anonymous, 2018)

In the following reaction, it interacts with the NAD^+ .As a result, episterone is occurred by two different enzymes. After that, this component is interacted with the NAD^+ and H^+, then episterol occurs. The reaction is catalyzed by 3-oxosteroid reductase and encoded by the *SDR* gene (Sawai et al, 2014). In the following reaction, episterol is reacted, and afterwards, ergosta-5,7,24(28)-trien-3β-ol is occurred compound. Here is catalyzed by two different enzymes such as delta 7-sterol-C5-desaturase and sterol C-5 desaturase. Also, this reaction is encoded by two *DWF7* and *ERG3* genes (Brumfield et al, 2010; Husselstein et al, 1999). In the following step, the ergosta-5,7,24(28)-trien-3β-ol interacts with the NAD^+ and H^+. As a result, 24-methylenecholesterol is synthesized. The enzyme Sterol Delta 7 Reductase catalyzes this process. The sterol delta 7 reductase is encoded by *OeDWF5* which is mapped between 18,685,600 and 18,689,318 positions on the olive genome (pmn.plantcyc.org). The *OeDWF5* is also a genome size of 3719 bp (Lecain et al, 1996). The *OeDWF5* converts 5-dehydroavenasterol to isofucosterole, ergosta-5,7,24 (28) -trien-3β-ol into 24-methylenecholesterole (Clouse, 2002; Benveniste, 2004). In the following reaction, the 24-methylenecholesterol is reacted, then it is transformed into 24-methyldesmosterol.

The reaction is catalyzed by (Knoch et al., 2018) isofucosterol isomerase and, the isofucosterol isomerase is encoded by *DWF1*. It is also a genome size of 2699 bp. The study reported that the *DWF1* is provided with a reduction in the isomerization as well as of the Δ24(28)-bonds of sterol, more correctly that of 24-methylenecholesterol and isofucosterol (Klahre et al., 1998). The isofucosterol isomerase catalyzes the reaction and is encoded by the *DWF1* (Kushiro et al., 2001). Finally, the 24-methyldesmosterol interacts with NADPH and H^+, and the campesterol is formed (Figure 12).

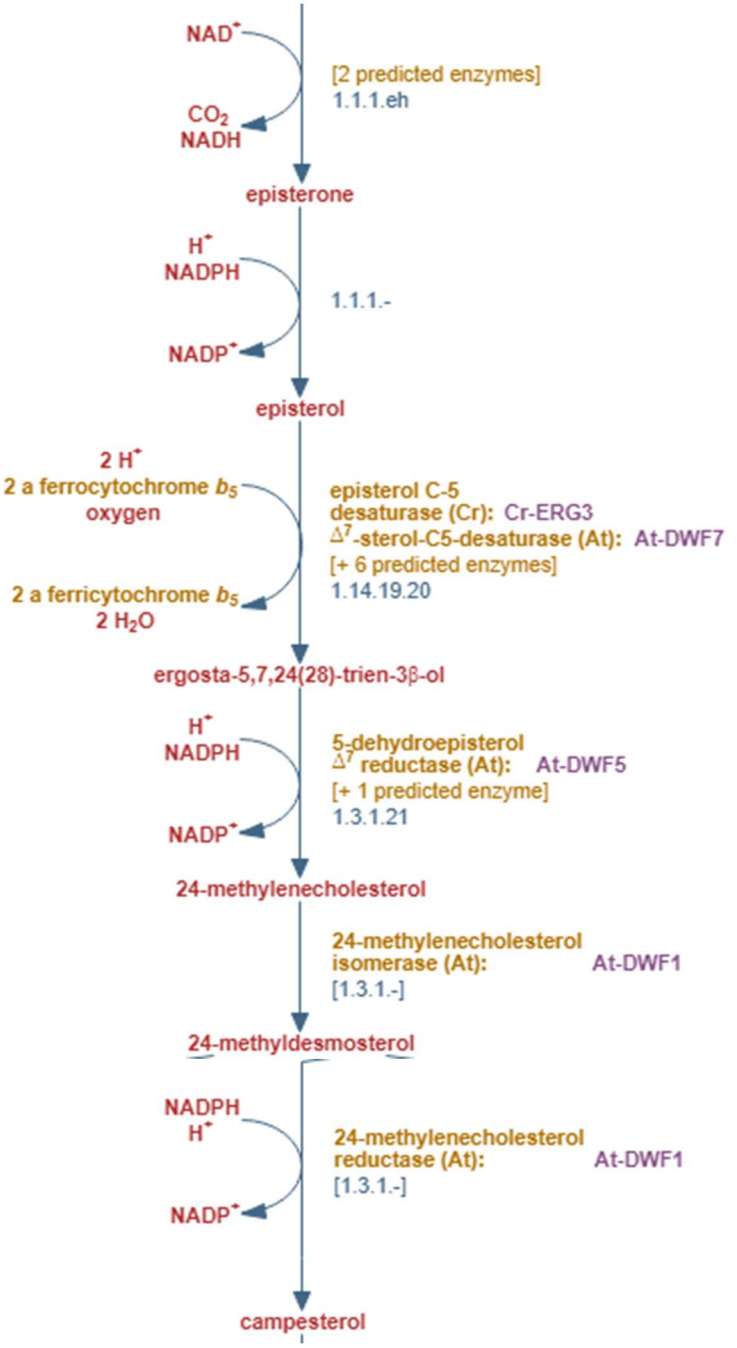


Figure 12. Synthesis of Campesterol (Anonymous, 2018)

1.3. Aims of The Study of The Thesis

Phytosterols are one of the vital molecules for plant development and have beneficial impacts on human health. For that reason, in thesis, we investigated the phytosterol biosynthesis pathway in olives. In *Arabidopsis*, the pathway contains various enzymes encoding by *DWF1, DWF2, SMT1, SMT2.1, DWF7* and *OeDWF5* genes (https://www.arabidopsis.org/). This study aims to analyze expression levels of the *OeDWF5* gene in olive fruits at eight different ripening periods. We designed primers and determined their efficiency for Real Time PCR analysis. The phytosterol synthesize in various olive tissues, including seed, root, stem, leaves, and fruits, in different concentrations (Tasan et al., 2006). They can also help plants to adapt to changing environmental conditions and high temperatures (Jiwan ve ark., 1993). The phytosterols compositions, forms, and structures may depend on environmental and genetic factors. Those differences may also cause different bioactivity specific to plant species. Therefore, determining phytosterol content in different plant species is crucial for plant development and health. The plant derived phytosterols are composed of free sterols, fatty acid esters, glycoside, and fatty acid glycoside (Moreau et al., 2002). However, the phytosterol biosynthetic pathways are not determined in olive. *OeDWF5* enzyme converts 5-dehydroavenasterol to isofucosterole, ergosta-5,7,24 (28) -trien-3β-ol into 24-methylenecholesterole (Clouse, 2002) and (Benveniste, 2004).

CHAPTER 2
PREVIOUS STUDIES

Phytosterol is a significant compound for organisms. Therefore, it has been an object of research since the 1920s. Furthermore, the olive is critical of the plant that a broadly of plant growth in the world. Hence, it has been continuing to research in the science world. Furthermore, that is significant in wellness, and mythological areas. Wang et al, (2019) showed that Brassinosteroids (BRs) are steroid hormones that recreate wide roles in plant growth and development. Therefore, the characterization of several BR-related genes in the Moso bamboo genome (*Phyllostachys edulis*) was reported. These genes have the *OeDWF5*. As a result, thirty-four positive and three negative co-expression modules were identified by 44 candidate genes in the recently developing bamboo shoot. According to the findings, gene expression patterns and co-expression modules of BR action-related in bamboo shoots could enhance bamboo development via BR biosynthesis and signal transduction mechanism. This consequence is the first part toward the cloning and functional examination of the function of the BR action-related genes in it.

Ramadan et al, (2019) indicated that the *Calotropis procera* is a vital plant for pharmaceuticals. This plant contains immunostimulant β- Sitosterol (BS). This study investigated the effect of day length before and after irrigation on the BS accumulation pathway, namely, phytosterol biosynthesis. Studying the enzymes in BS biosynthetic pathway indicated the upregulation at dawn and predusk of the SMT2 and SMO2 genes encoding sterol methyltransferase two and methyl sterol monooxygenase, two critical enzymes in BS accumulation in this plant. For that reason, there is no regulation at the different time points of the CYP710A gene encoding sterol 22-desaturase, an enzyme that exhausting β- Sitosterol towards the biosynthesis of Stigmasterol compound. As a result, the BS increase is huge at dawn when dehydrated and in good condition.

Kumar et al. (2018) phytosterols are vital to the plant membrane. The levels of the critical the phytosterol, this study has shown that β- Sitosterol in drought-tolerant rice seedlings are found to expand proportionally to the severity of drought stress. The HMG-CoA reductase is considered to play an essential function in the phytosterol biosynthesis. This investigation indicated that levels of drought stress are shown to increase proportionately with the HMG-CoA reductase of activity.

Leet al. (2004) roots of the *Panax ginseng,* it is well-known as the medical plant. In addition, it is found to contain phytosterol as well as bioactive triterpene saponins. Therefore, the study investigated the regulatory position of the Panax ginseng squalene synthesis (*PgSS1)* on the phytosterol biosynthesis of both triterpene saponins. The results have shown that the *PgSS1* is an essential role regulatory enzyme is not just for the phytosterol, nevertheless for triterpene biosynthesis and overexpressing of the *PgSS1* confers the hyperproduction of triterpene saponins to the *P. ginseng.* Han et al. (2010) in the phytosterol and triterpenoid saponin biosynthesis pathway, the squalene epoxidase is vital of the enzyme, because it plays a role in rate-limiting. In study has been researched the important role of two genes (PgSQE1 and PgSQE2) in the phytosterol and triterpene biosynthesis it. The result showed that these two are regulated in a distinct method, the PgSQE1 from these genes will regulate the ginsenoside biosynthesis. But also, the *P. ginseng* is shown no regulate phytosterols.

CHAPTER 3
MATERIALS AND METHODS

3.1. Plant Samples

In this thesis, four different olive varieties including Ayvalık, Memecik, Gemlik, and Gökçeada were used. Ayvalık, Gemlik and Memecik varieties were procured from the Directorate of Olive Research Station (Edremit, Balıkesir) managed by the Republic of Türkiye Ministry of Agriculture and Forestry. Also, Gökçeada variety was obtained from Gökçeada Island. Samples have been acquired from healthy olive trees and are collected for eight periods between the range of 15 days intervals for four months. The fruits were frozen immediately in liquid nitrogen (Table 1) and kept at -80 C until its used.

Table 1

Dates of acquiring in the sample's olive

Periods	Dates
1	01.07.2019
2	16.07.2019
3	02.08.2019
4	17.08.2019
5	03.09.2019
6	18.09.2019
7	03.10.2019
8	18.10.2019

3.2. Isolation of the Total RNAs

The frozen olive fruit samples were ground with a mortar and pestle in liquid nitrogen. The plastic materials used in this process were incubated into a solution containing 1% DEPC overnight, then sterilized at 121°C for 15 minutes. Sterile packets of RNase-free microcentrifuge tubes and micropipette tips are used. The total RNAs were isolated with Trizol (Ambion-15596-02) and a plant tissues-specific RNA isolation kit (PureLink® RNA Mini Kit (Invitrogen-12183025). In the method, the following steps were performed, respectively.

- First, the olive fruits were crushed with the help of a pestle to mesocarp tissue. Afterward, the endocarp with seeds was removed.
- Secondly, 100 mg of the mesocarp tissue was transferred to the microcentrifuge tube.
- 1 ml of the Trizol solution was added to the tissue, mixed, and incubated at room temperature for 5 minutes.
- 200 µl chloroform were added to the samples and mixed with hands for 15s, then incubated at room temperature for 3 min.
- Then, these samples were precipitated by a centrifuge using 12.000 g for 15 min.
- The upper liquid phase was transferred to the new microcentrifuge tube after the centrifugation. An equal volume (400 µl) of %70 ethanol was added to each tube and mixed with a vortex.
- The samples (700 µl) were transferred to the spin cartridge tube, and the tube was centrifuged at 12.000 g for 15 min.
- After centrifugation, the wash buffer I (700 µl) was added to samples, then the remaining liquid was discarded, and the last two steps are repeated.
- The examples were centrifuged at 12,000 × g for 15 seconds at room temperature. Afterward, samples inside the spin cartridge tube were transformed into a new spin cartridge tube.
- 500 µl of Wash Buffer II was added to the tubes.
- It was centrifuged at 12,000 × g for 15 seconds at room temperature, and the lower liquid was discarded.
- The last two steps were repeated.
- The samples were centrifuged at 14, 000 g for 3 minutes, then samples were transformed into the spin cartridge tube.
- Afterward, 50 µl of RNase-free water was added to the center of the spin cartridge and incubated at room temperature for 3 minutes.
- Finally, these samples were centrifugal to the power of 12.000 g for 2 min. It was stored at -80° C.

3.3. Measurement of the RNA

The RNA amounts were measured using a Qubit fluorometer (Invitrogen, Q32866). The measurements were performed in conformance with the device's instructions. To accomplish this, 199 µl of reaction buffer was combined with 1 µl of RNA Reagent (Invitrogen-Q10211). After that, 1 µl of the mixture was removed and replaced with 1 µl of the RNA sample. The samples were read in the device after being preserved at room temperature for approximately two minutes, and their concentration levels in ng/ µl were calculated.

3.4. Selection of Primers in the Study

In the first period of this thesis, to carry out gene expression studies, protein, transcript, and genomic sequences of the phytosterol biosynthesis *OeDWF5* gene were determined in the existing olive genomes. Afterwards, primers specific to the obtained transcript sequences were designed and reaction conditions were optimized in olive fruit tissues. The NCBI primer blast program was used to select the primers. The NCBI accession codes of the obtained transcript sequences were used as input. In the selection of primers, the parameters were identified such that the optimum value of T_m was between 60 ℃ and 58 ℃ and 62 ℃, T_m difference in the rang primers was at most 1 ℃, PCR product size was in the range of 80-150 base pairs. In addition, if the transcript sequences do not consist of a single exon, at least one of the primers is bound to the junction of two exons.

Primers with a GC content of 40-60 %, having a melting temperature at 59.1°C, and producing amplicons that are 70-150 bp long were designed for Real-Time PCR.

Table 2

Nucleotide sequence of the primers

Name Primer	Sequence Primer (5'-3')	Temperature of Melting
*OeDWF5*_ Forward_primer	AGCATTTGAGGCTGCACTTC	59.1
*OeDWF5*_Reverse_primer	ACACCATTTGCCTTGTATACGG	59.1

3.5. DNase Treatmets

DNase treatments prevent DNA contaminations. cDNAs synthesis, the RNA was treated with DNase 5 µl total RNA was used in the reactions, and the DNase enzyme was inactivated by temperatures at 65 °C for 10 minutes (Table 3).

Table 3

Components of the Reaction of the DNase

	Amount	Final Concentration
10 × Buffer reaction	1 µl	1×
RNA	1000 ng	100 ng / µl
DNase I (1 µ / µl)	1 µl	0.1 µ / µl
Water	-	-
Volume last	10 µl	-

3.6. Synthesis of the cDNA

In the study, the synthesis of cDNA is performed with the help of the kit (43688814; Applied Biosystems). In this method, the following procedure was performed, respectively (Table 4).

Table 4

cDNA synthesis mix used in quantitative PCR studies

The Reaction Complement	Amount
10× Reaction Buffer	2 µl
25×dNTP mix	0.8 µl
10× RT Random	2 µl
Reverse Transcription	1 µl
RNase Inhibitor	1 µl
Nuclease-Free Water	3.2 µl
Total Volume	10 µl

The High-Capacity cDNA Reverse Transcription Kit's manufacturer's instructions were performed in a series of reverse transcription reactions to produce cDNA. The PCR products were kept at +4 °C until they were used. Following that, the DNase-treated RNA sample was mixed with the cDNA reaction mixture and incubated according to the program as shown Table 5.

Table 5

The cDNA synthesis program for PCR studies

Process of Action	Temperature (°C)	Time
1.	25	10 min
2.	37	120 min
3.	85	5 min
4.	4	Endless (∞)

3.7. The Real-Time PCR

The expression levels of the olive *OeDWF5* gene were selected by gene expression using SYBR-Green with the RT-PCR. Reactions in the RT-PCR, it has been made 1 µl cDNA, 5 µl SYBER-Green master mix and 4 µl nuclease-free water add to the 96 well plates. Also, the study has established control reactions in reverse transcription and negative control reactions to check for likely DNA or RNA contamination. Studies of gene expression have been performed on devices with the Applied Biosystems 7500. Clathrin and Pkaba are used as reference genes in this thesis. Three biological and technical repetitions have been made for each gene that has been used. Each plate contained one target gene and two reference genes.

3.8. The Standard Curve Experiments Establishment

In the study, the Real-Time PCR for standard curve drawing for selected primers has been consisted of. This reaction was used as a pattern from the olive fruit cDNA, which purified the PCR product carrying regions to the primers that will be attached. The estimated number of copies of the PCR product was calculated according to the formula "Number of copies = (The DNA amount $*6.022 \times 10^{23}$) / (The DNA sequence length $*1 \times 10^{9}*650$)". (Dorak, 2006).

Ten copies as a minor dilution to use patterns have performed dilution six series. Reactions with the SYBR GREEN technique established at the relevant dilution points and results in Ct were evaluated for efficiency.

3.9. Buffers Used and Agarose Gel Electrophoresis

3.9.1. Agarose Gel Electrophoresis

The study performed %0.1 agarose gel electrophoresis products of the PCR and RNA samples viewing. First, the 50 ml 1X TAE buffer including 0,5 gr agarose of heating was provided as a homogeneous solution. Afterwards, this gel was added ethidium bromide 3 µl into the gel tray spilt. After this process, added 6X ladder loading paint and the wells in the order specified were loaded.

The gel of the first wells loaded a 1 kb ladder (Fermentas DNA ladder SM0633). Finally, the gel was conducted at 6,5 V /cm. The UV of the PCR products viewed, photo machine Olympus C-5060 has been recorded.

3.9.2. The Buffer 10XTAE

The buffer 10X TAE to prepare was added to use chemicals of the 48,4 gr Tris base, 3,72 gr EDTA and thawed into the 300 ml dH2O. Then, the pH 8,0 with acetic acid was brought. Finally, it was preserved at room temperature.

3.9.3. The Buffer 1 X TAE

The 100 ml of 10X TAE earlier prepare into the 900 ml dH2O was added.

3.9.4. Stok Solution of the Ethidium Bromide

The ethidium bromide 0,1 gr in 10 ml dH2O was thawed. Finally, this solution was preserved at room temperature.

CHAPTER 4
RESULTS AND DISCUSSION

4.1. Results

4.1.1. The RNA Isolation

Total RNAs isolated from the mesocarp tissues were checked on 1,5 % agarose gel electrophoresis. The presence of ribosomal RNAs (such as 28s and 18s) without degradation was observed for each sample (Figure 13,14,15 and 16). RNA concentrations were measured between 176 and 1072 ng/µl (Table 6).

Table 6

Measurement of RNA samples used in the study by a fluorometric method

Sample the RNA	Concentration (ng/µl)
01.07.2019 (Period 1)	
Ayvalık	480
Memecik	436
Gemlik	500
Gökçeada	554
16.07.2019 (Period 2)	
Ayvalık	310
Memecik	596
Gemlik	324
Gökçeada	260

Table 6 (continued)

02.08.2019 (Period 3)	
Ayvalık	590
Memecik	736
Gemlik	348
Gökçeada	352
17.08.2019 (Period 4)	
Ayvalık	554
Memecik	666
Gemlik	348
Gökçeada	342
03.09.2019 (Period 5)	
Ayvalık	252
Memecik	808
Gemlik	378
Gökçeada	176
18.09.2019 (Period 6)	
Ayvalık	636
Memecik	1072
Gemlik	668
Gökçeada	352
03.10.2019 (Period 7)	
Ayvalık	438
Memecik	618
Gemlik	406
Gökçeada	240

Table 6 (continued)

18.10.2019 (Period 8)

Ayvalık	262
Memecik	586
Gemlik	282
Gökçeada	350

In the first period, the highest amount of RNA was obtained from Gökçeada (554 ng/µl) and, lowest in Memecik (436 ng/µl). In the 2. Period, RNA concentration was high for Memecik (596 ng/µl) and low for Gemlik (324 ng/µl). In the third period, RNA concentration was 736 ng/µl for Memecik and 590 ng/µl for Ayvalık. The concentration of the latest periods was generally high but low at the final. The results show a correlation between fruit size and ripening periods.

Olive varieties have a difference in mRNA concentration because of their unique fruits and ripening processes.

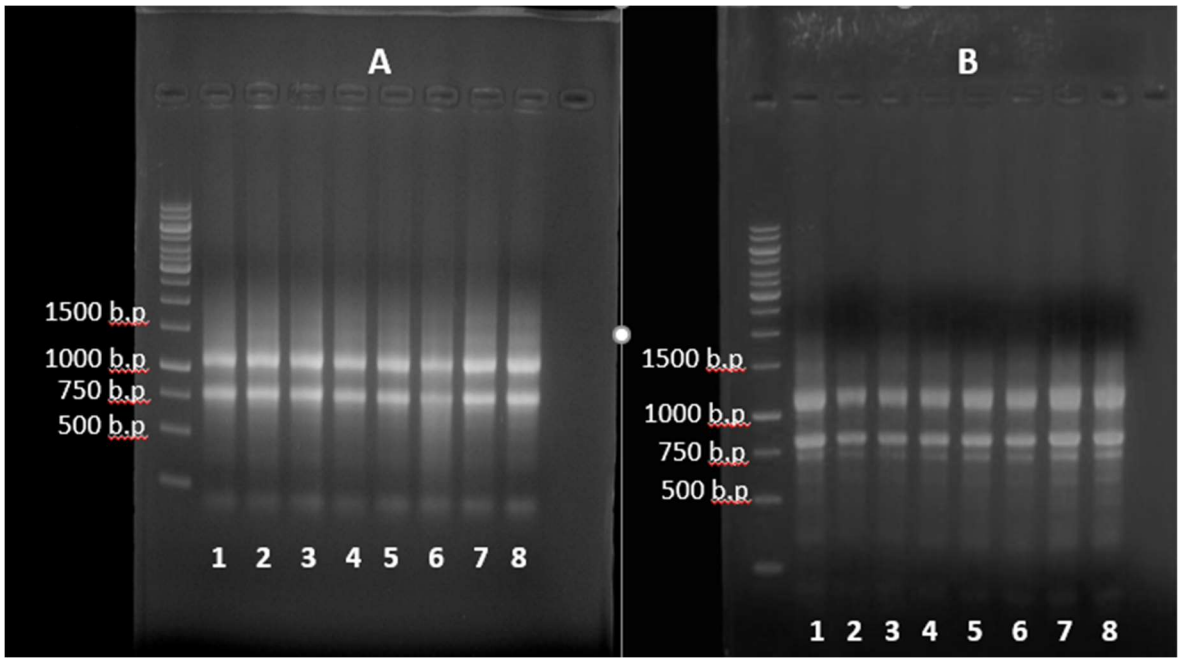


Figure 13. Agarose gel electrophoresis images of the RNA samples used in the study. A, in periods 1 and B, in periods 2. 1,2 the Ayvalık, 3,4 the Gemlik, 5,6 the Gökçeada and 7,8 the Memecik

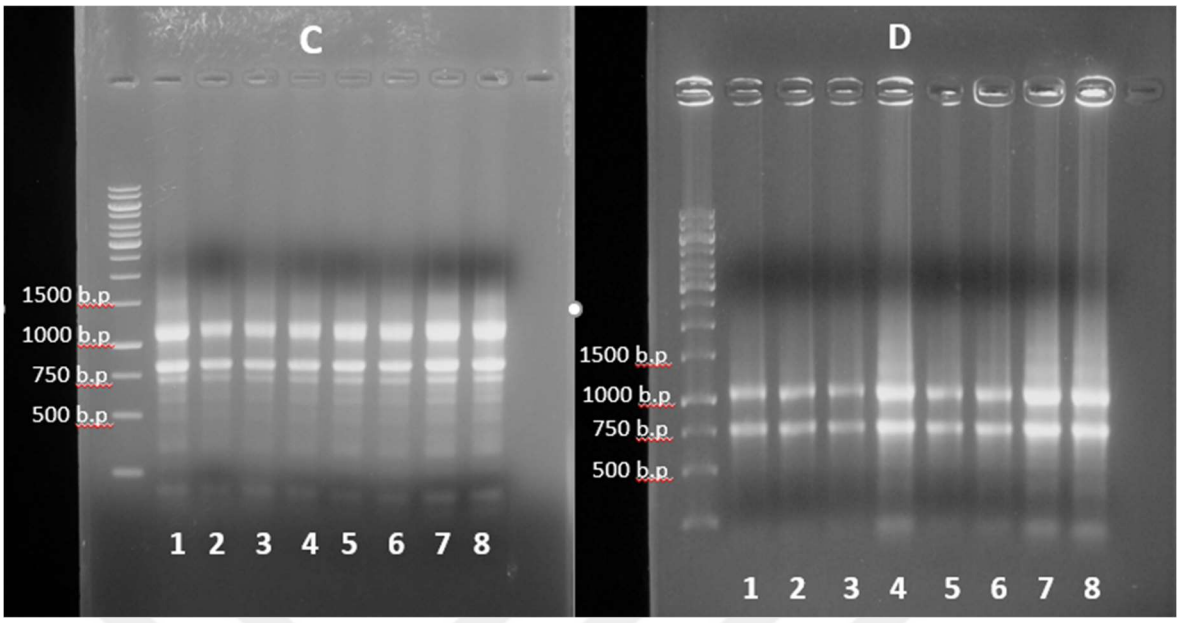


Figure 14. Agarose gel electrophoresis images of the RNA samples used in the study. C, in periods 3 and D, in periods 4. 1,2 the Ayvalık, 3,4 the Gemlik, 5,6 the Gökçeada and 7,8 the Memecik

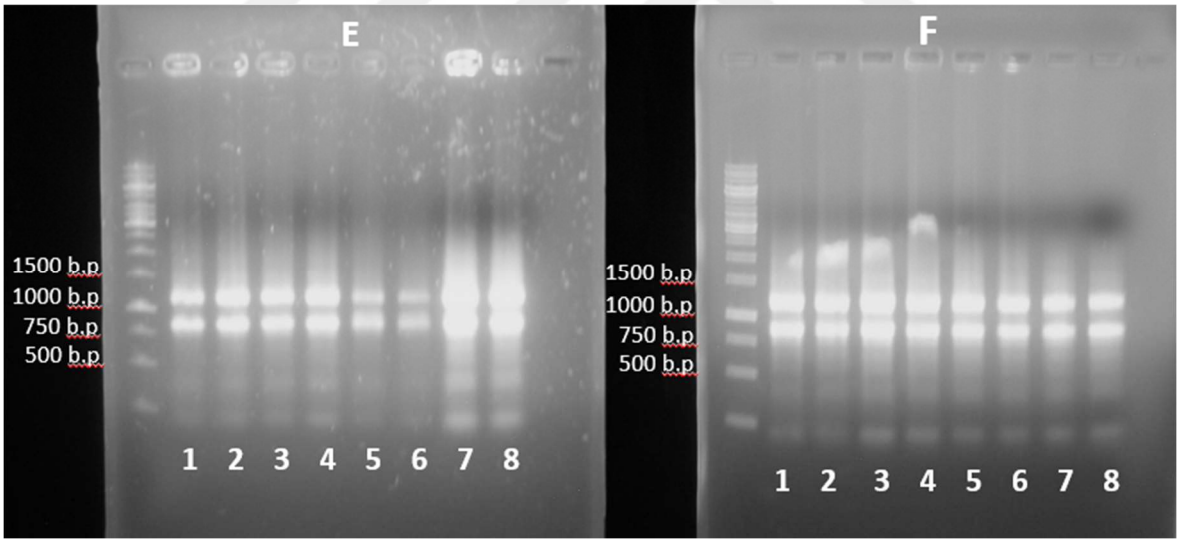


Figure 15**.** Agarose gel electrophoresis images of the RNA samples used in the study. E, in periods 5 and F, in periods 6. 1,2 the Ayvalık, 3,4 the Gemlik, 5,6 the Gökçeada and 7,8 the Memecik

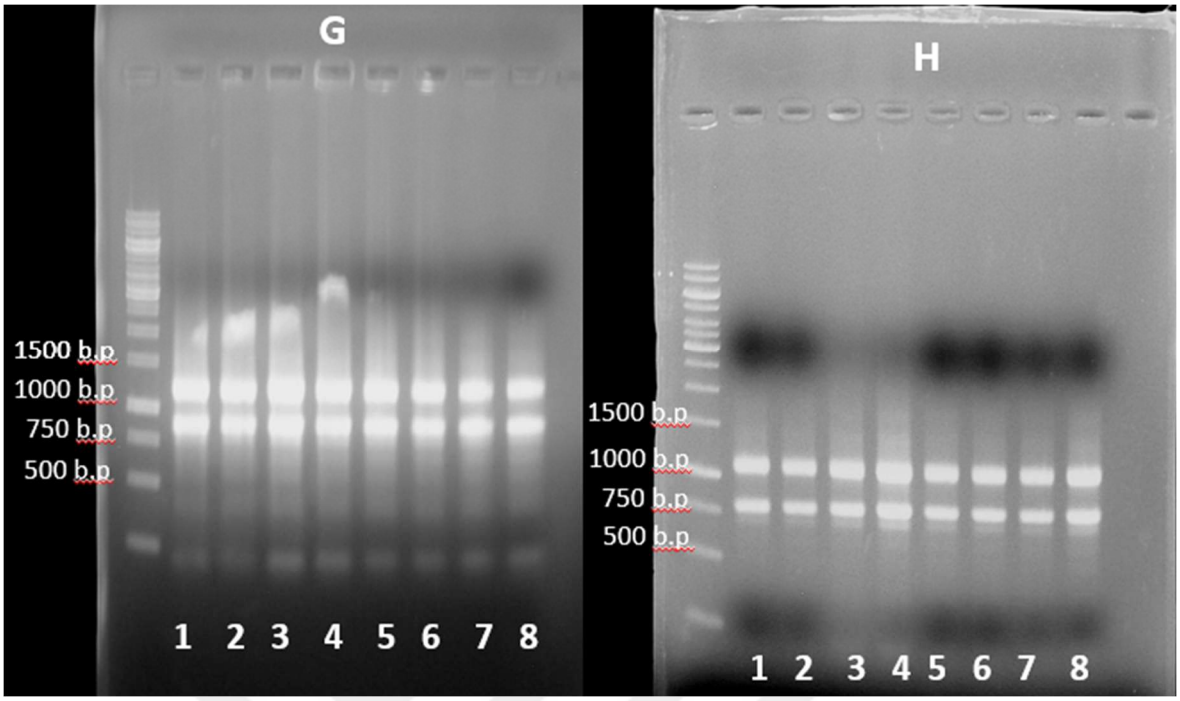


Figure 16. Agarose gel electrophoresis images of the RNA samples used in the study. G, in periods 7 and H, in periods 8. 1,2 the Ayvalık, 3,4 the Gemlik, 5,6 the Gökçeada and 7,8 the Memecik.

For the synthesis of the cDNA, 100 ng of the RNA template has been used. RNA was not degraded in samples of examining photographs gel.

4.1.2. Studies of Gene Expression

The SYBR-Green based qPCR technique was used to investigate the expression of the *OeDWF5* gene. qPCR is an extremely reliable and valuable method that utilizes fluorescently labeled probes as reporters for target genes.

The efficiencies of primers selected for the gene expression studies were calculated and a standard curve was drawn. Afterwards, the expression levels were compared with the comparative Ct method under the conditions in the appropriate efficiency range.

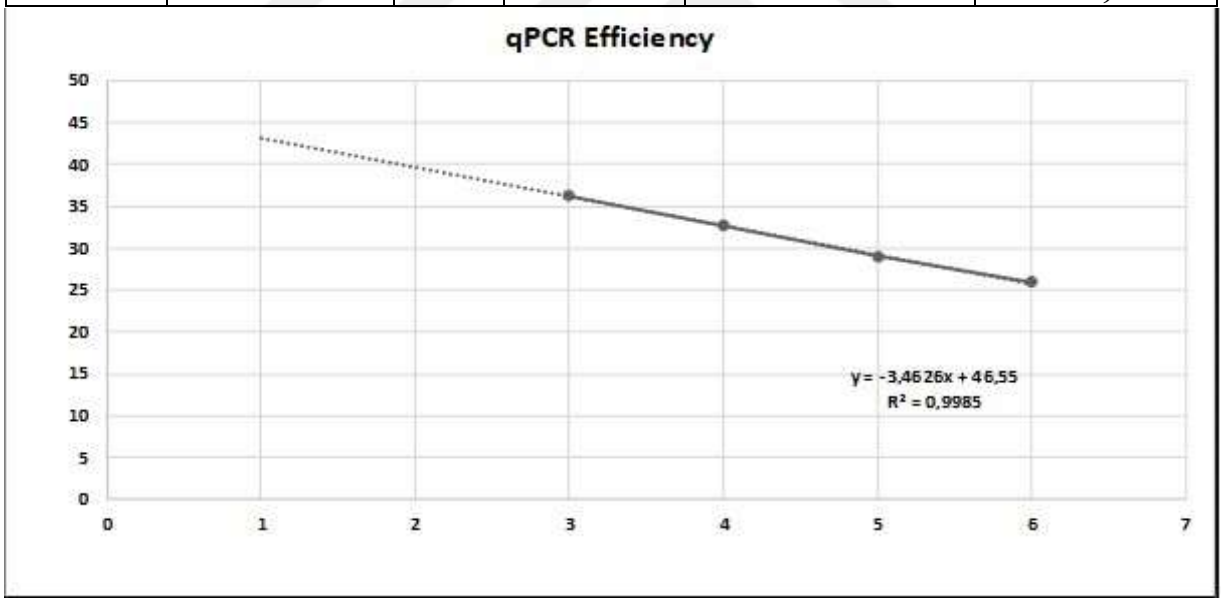
4.1.3. Efficiencies of Primers

The slope of the relevant line was calculated with obtained Ct values as the logarithm of the estimated copy numbers. To calculate the PCR efficiency, the slope value was used by the formula "Efficiency= $(-1+10^{(-\frac{1}{slope})}\times 100)$" (Dorak, 2006). The efficiency of primer pairs selected for the SYBR Green-based qPCR analysis is calculated with standard curve experiments (Table 7). When the standard curve results for the *OeDWF5* primers pairs are examined, the R^2 value was calculated as 0.9985, and data belonging to points of serial dilutions acquired has been understood to be linear enough. When the slope of this line was situated in the -3.462598801 formula, it was calculated that the PCR reaction operates with an efficiency of 94.4%. The primer and reaction condition selected for the *OeDWF5* gene was suitable for expression operations (Table 7.).

Table 7.

The curve of efficiency of the *OeDWF5* gene and efficiency values

Dilution		Log	Ct values	Dilution of coefficient	10
1	1000000	6	25,9302025	Dilution of number	6
2	100000	5	28,9955239		
3	10000	4	32,7180462	Target =	*OeDWF5*.2
4	1000	3	36.2313576	Sample =	Olive fruit cDNA
5	100	2		Slope =	-3.462598801
6	10	1		Efficiency (%) =	**94,4**



4.1.4. Gene Expression Studies

The expression level *OeDWF5* were investigated in 4 olive genotypes cultivated in Edremit and Gökçeada. A reaction with an observed reproduction of less than 37 Ct values in negative control reactions, the product with more than the T_m value of the product observed, and a 0.5 Ct difference between technical and observed are ignored and repeated at this stage. These findings demonstrated that negative control did not exist in reproduction. Furthermore, melt curve analyses have been interpreted as a specific replication of the *OeDWF5* gene's product (Figure 17).

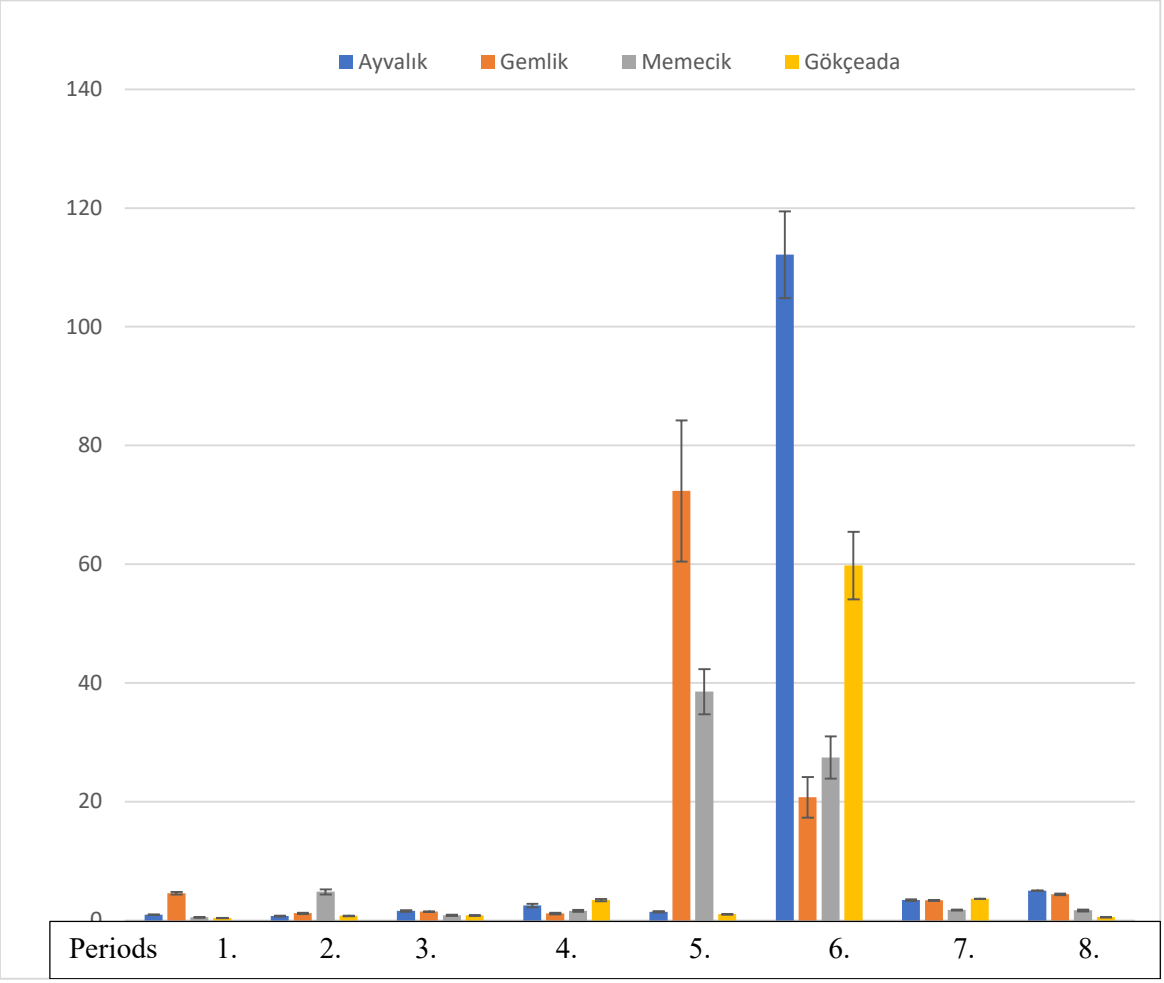


Figure 17. The Real-Time PCR results of the *OeDWF5* gene

The olive fruits were harvested at eight different ripening periods between July to November. According to our results, the expression levels of *OeDWF5* were low and there

is no significant difference between gene expressions until the 5th period. The expression of *OeDWF5* was first observed for Gemlik and Memecik genotypes at the ripening period 5 which corresponds 03.09.2019. At this period, the expression levels of *OeDWF5* in Gemlik were almost 2 times higher than Memecik. It was observed that the gene expression of Ayvalık and Gökçeada cultivars was low in *OeDWF5.* At the ripening stage 6, *OeDWF5* were expressed in all genotypes with the highest expression in Ayvalık following Gökçeada, Memecik and Gemlik with lower levels. There was no statistically vital variation among olive genotypes and the expression levels of *OeDWF5* in ripening periods 7 and 8.

The *OeDWF5* gene is vital for all plants because it is involved in the growth and development. Ashwagandha (*Withania somnifera* Dunal. Linn*.)* is vital to human health because it was used against tumor and tubercular glands. Plant growth regulators (PGRs) were applied in this study on Ashwagandha, and results were investigated. This study used Ethrel, Paclobutrazol (PBZ), Succinic Acid (SucA), Salicylic Acid (SA), and Methyl Jasmonate (MJA). Article show that expression levels of the *DWF5* were expressed in PGRs the highest expression in following MJA, Ethrel and SucA. The expression of *DWF5* was the lowest in the SA (Kalariya et al.,2020). Our study of the expression of levels *DWF5* investigated different ripening periods. Our research found that expression levels varied across eight different ripening periods. Our results showed that gene expression levels of the *DWF5* is not statistically significant, especially during early ripening periods. By contrast, maximum gene expression was occurred in the 5th and 6th periods. In the 5th periods, the highest gene expression was in the Gemlik cultivars. In the 6th periods, all cultivars were occurred gene expression, respectively, Ayvalık, Gökçeada, Memecik, and Gemlik. The study indicated that there was not mathematically difference in other periods.

This investigation involved a comparative analysis of the phytosterol in *olive (Olea europaea L.)* from 21 different varieties and 19 varieties of rapeseed (*Brassica napus L.*) from the 2004-2005 and 2005-2006 ripening periods. Results indicated that for rapeseed phytosterol was the highest content in Sitosterol, following by Campesterol and Brassicasterol. Other analysis results showed that the content of olive Sitosterol and following by avenasterol (Şeker and Gül, 2006). Our thesis was not examined of the phytosterol content. It will serve as the foundation for future research in this area.

The phytosterol contents of cultivars Arbequina, Ascolano, Frantoio, Koronelki, Manzanillo, Mission, and Sevillano olive cultivars were compared in this study. The contents

of Sitosterol, Campesterol and Stigmasterol differed according to the cultivars. There was no statistically meaningful difference in Stigmasterol and Campesterol contents in all olive cultivars. Sitosterol was identified in all genotypes, with Mission followed by Sevillano, Kalamata, Manzanillo, Frantoio, and Arbequire, all of which had lover levels (Zhou, 2012). Our study was not conducted to detect the content of Sitosterol, Campesterol, and Stigmasterol. On the other hand, we studied four different varieties. *OeDWF5* gene expressions from this study can be studied.

CHAPTER 5
CONCLUSION

In this thesis, the expression levels of *OeDWF5* were investigated over eight different time periods, and four different olive cultivars. We used Ayvalık, Gemlik, Memecik, and Gökçeada. The results revealed that gene expression levels of the *OeDWF5* vary depending on genotypes and ripening periods.

The dissertation demonstrated that phytosterol biosynthesis has altered over time due to changes in the rate of synthesis during the ripening of olives. The findings of this study indicate that if high phytosterol -content fruits are desired, they should be harvested in the 5^{th} and 6^{th} periods. In the 5^{th} periods, Gemlik and Memecik cultivars should be harvested. In the 6^{th} periods, Ayvalık, Memecik, Gemlik, and Gökçeada cultivars can be harvested. At other periods, the amount of mRNA has decreased.

REFERENCES

Andjelkovic, M., Acun, S., Van Hoed, V., Verhé, R., & Van Camp, J. (2009). Chemical composition of Turkish olive oil—Ayvalik. Journal of the American Oil Chemists' Society, 86(2), 135-140.

Anonymous, (2018). PlantCyc Pathway: Phytosterol biosynthesis (Plants) https://pmn.plantcyc.org/PLANT/NEW-IMAGE?type=PATHWAY&object=PWY-2541&detail-level=4 (Date of access: 18.05.2018)

Anonymous, (2020).https://pubchem.ncbi.nlm.nih.gov/compound/5997#section=Structures (Date of access: 17.12.2020)

Anonymous, (2021). National Library of Medicine https://pubchem.ncbi.nlm.nih.gov/#query=stigmasterol%2C%20sitosterol%20and%20campesterol (Date of access: 16.01.2021)

Anonymous, (2023). Food and Agriculture Organization. https://fenix.fao.org/faostat/internal/en/?#data/QCL (Date of access: 01.01.2023)

AZİMİ, Mahmoud, et al. "Gemlik Zeytin Çeşidi Üzerine Farklı Aşı Kombinasyonlarında Besin Maddesi Alımı." Zeytin Bilimi 5.1: 1-8.

Brumfield, K. M., Moroney, J. V., Moore, T. S., Simms, T. A., & Donze, D. (2010). Functional characterization of the Chlamydomonas reinhardtii ERG3 ortholog, a gene involved in the biosynthesis of ergosterol. PLoS One, 5(1).

Canözer, Ö. (1991). Standart zeytin çeşitleri kataloğu. TC Tarım ve Köyişleri Bakanlığı, Mesleki Yayınlar Serisi, Genel, (334).

Carland, F., Fujioka, S., & Nelson, T. (2010). The sterol methyltransferases SMT1, SMT2, and SMT3 influence Arabidopsis development through nonbrassinosteroid products. Plant physiology, 153(2), 741-756.

Choe, S., Tanaka, A., Noguchi, T., Fujioka, S., Takatsuto, S., Ross, A. S., ... & Feldmann, K. A. (2000). Lesions in the sterol Δ7 reductase gene of Arabidopsis cause dwarfism due to a block in brassinosteroid biosynthesis. The Plant Journal, 21(5), 431-443.

Clouse, S. D. (2002). Arabidopsis mutants reveal multiple roles for sterols in plant development. The Plant Cell, 14(9), 1995-2000.

Dağdelen, A., Tümen, G., Özcan, M. M., & Dündar, E. (2013). Phenolics profiles of olive fruits (Olea europaea L.) and oils from Ayvalık, Domat and Gemlik varieties at different ripening stages. Food chemistry, 136(1), 41-45.

Darnet, S., & Rahier, A. (2004). Plant sterol biosynthesis: identification of two distinct families of sterol 4alpha-methyl oxidases. *Biochemical Journal*, *378*(3), 889-898.

De Jong, A., Plat, J., & Mensink, R. P. (2003). Metabolic effects of plant sterols and stanols. The Journal of Nutritional Biochemistry, 14(7), 362-369.

Dennis, A. L., & Nes, W. D. (2002). Sterol methyl transferase. Evidence for successive C-methyl transfer reactions generating Δ24 (28)-and Δ25 (27)-olefins by a single plant enzyme. Tetrahedron letters, 43(39), 7017-7021.

Dufourc, E. J. (2008). The role of phytosterols in plant adaptation to temperature. Plant signaling & behavior, 3(2), 133-134.

El Yamani, M., Boussakouran, A., & Rharrabti, Y. (2020). Leaf water status, physiological behavior and biochemical mechanism involved in young olive plants under water deficit. Scientia Horticulturae, 261, 108906.

Feldman, M. J. (2012). *Identifying regulatory components of phytosterol biosynthesis in the genomics era*. Washington State University.

Fernández-Poyatos, M. P., Ruiz-Medina, A., & Llorent-Martínez, E. J. (2019). Phytochemical profile, mineral content, and antioxidant activity of Olea europaea L. cv. Cornezuelo table olives. Influence of in vitro simulated gastrointestinal digestion. Food chemistry, 297, 124933.

Fu, J., Liu, G., Yang, M., Wang, X., Chen, X., Chen, F., & Yang, Y. (2019). Isolation and functional analysis of squalene synthase gene in tea plant Camellia sinensis. Plant Physiology and Biochemistry, 142, 53-58.

Green, P. S. (2002). A revision of Olea L.(Oleaceae). Kew Bulletin, 91-140.

Gül, M. K., & Şeker, M. (2006). Comparative analysis of phytosterol components from rapeseed (Brassica napus L.) and olive (Olea europaea L.) varieties. *European Journal of Lipid Science and Technology*, *108*(9), 759-765.

Han, J. Y., In, J. G., Kwon, Y. S., & Choi, Y. E. (2010). Regulation of ginsenoside and phytosterol biosynthesis by RNA interferences of squalene epoxidase gene in Panax ginseng. Phytochemistry, 71(1), 36-46.

Hannich, J. Thomas, Kyohei Umebayashi, and Howard Riezman (2011) "Distribution and functions of sterols and sphingolipids." Cold Spring Harbor perspectives in biology 3.5: a004762.

Husselstein, T., Schaller, H., Gachotte, D., & Benveniste, P. (1999). Δ7-sterol-C5-desaturase: molecular characterization and functional expression of wild-type and mutant alleles. Plant molecular biology, 39(5), 891-906.

Inês, C., Corbacho, J., Paredes, M. A., Labrador, J., Cordeiro, A. M., & Gomez-Jimenez, M. C. (2019). Regulation of sterol content and biosynthetic gene expression during flower opening and early fruit development in olive. Physiologia plantarum, 167(4), 526-539.

Jain, S. M., & Gupta, P. K. (Eds.). (2005). Protocol for somatic embryogenesis in woody plants (Vol. 77). Dordrecht: Springer.

Jaramillo-Madrid, A. C., Ashworth, J., Fabris, M., & Ralph, P. J. (2019). Phytosterol biosynthesis and production by diatoms (Bacillariophyceae). Phytochemistry, 163, 46-57.

Kalariya, K. A., Gajbhiye, N. A., Minipara, D., Saran, P. L., Kumar, S., Solanki, V., ... & Manivel, P. (2020). Altered biomass allocation and quality improvement in roots of Indian ginseng Withania somnifera Dunal. Linn. through physiological interventions. *Heliyon*, *6*(9), e05093.

Kayahan M. 1998. Lipidler. Gıda Kimyası, İ Saldamlı (Editör), 152-153 s, Hacettepe Yay., Ankara

Kıralan, Mustafa, and Ali Bayrak. "Oxidative and antiradical stabilities of two important virgin olive oils from Ayvalik and Memecik olive cultivars in Turkey." *International Journal of Food Properties* 16.3 (2013): 649-657.

Klahre, U., Noguchi, T., Fujioka, S., Takatsuto, S., Yokota, T., Nomura, T., ... & Chua, N. H. (1998). The Arabidopsis DIMINUTO/DWARF1 gene encodes a protein involved in steroid synthesis. The Plant Cell, 10(10), 1677-1690.

Knoch, E., Sugawara, S., Mori, T., Poulsen, C., Fukushima, A., Harholt, J., ... & Saito, K. (2018). Third DWF1 paralog in Solanaceae, sterol Δ24-isomerase, branches withanolide biosynthesis from the general phytosterol pathway. *Proceedings of the National Academy of Sciences*, *115*(34), E8096-E8103.

Küçükyaşar, S., & Pazır, F. (2019). Organik ve Konvansiyonel Memecik Çeşidi Yeşil Zeytinler Arasındaki Fiziksel, Kimyasal ve Pomolojik Özellikler Açısından Farklılıklar. Akademik Gıda, 17(1), 47-54.

Kumar, M. S., Ali, K., Dahuja, A., & Tyagi, A. (2015). Role of phytosterols in drought stress tolerance in rice. Plant Physiology and Biochemistry, 96, 83-89.

Kushiro, M., Nakano, T., Sato, K., Yamagishi, K., Asami, T., Nakano, A., ... & Yoshida, S. (2001). Obtusifoliol 14α-demethylase (CYP51) antisense Arabidopsis shows slow growth and long life. Biochemical and Biophysical Research Communications, 285(1), 98-104.

Lecain, E., Chenivesse, X., Spagnoli, R., & Pompon, D. (1996). Cloning by metabolic interference in yeast and enzymatic characterization of Arabidopsis thaliana sterol 7-reductase. Journal of Biological Chemistry, 271(18), 10866-10873.

Lee, M. H., Jeong, J. H., Seo, J. W., Shin, C. G., Kim, Y. S., In, J. G., ... & Choi, Y. E. (2004). Enhanced triterpene and phytosterol biosynthesis in Panax ginseng overexpressing squalene synthase gene. Plant and Cell Physiology, 45(8), 976-984.

Lu, Y., Zhou, W., Wei, L., Li, J., Jia, J., Li, F., ... & Xu, J. (2014). Regulation of the cholesterol biosynthetic pathway and its integration with fatty acid biosynthesis in the oleaginous microalga Nannochloropsis oceanica. Biotechnology for biofuels, 7(1), 81.

Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in lipid research*, *41*(6), 457-500.

Pascal, S., Taton, M., & Rahier, A. (1993). Plant sterol biosynthesis. Identification and characterization of two distinct microsomal oxidative enzymatic systems involved in sterol C4-demethylation. Journal of Biological Chemistry, 268(16), 11639-11654.

Ploier, B., Korber, M., Schmidt, C., Koch, B., Leitner, E., & Daum, G. (2015). Regulatory link between steryl ester formation and hydrolysis in the yeast Saccharomyces cerevisiae. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1851(7), 977-986.

Quilez, J., Garcia-Lorda, P., & Salas-Salvado, J. (2003). Potential uses and benefits of phytosterols in diet: present situation and future directions. Clinical Nutrition, 22(4), 343-351.

Rahier, A., Darnet, S., Bouvier, F., Camara, B., & Bard, M. (2006). Molecular and enzymatic characterizations of novel bifunctional 3β-hydroxysteroid dehydrogenases/C-4 decarboxylases from Arabidopsis thaliana. Journal of Biological Chemistry, 281(37), 27264-27277.

Rondet, S., Taton, M., & Rahier, A. (1999). Identification, Characterization, and Partial Purification of 4α-Carboxysterol–C3-Dehydrogenase/C4-Decarboxylase from Zea mays. Archives of biochemistry and biophysics, 366(2), 249-260.

Sawai, S., Ohyama, K., Yasumoto, S., Seki, H., Sakuma, T., Yamamoto, T., ... & Muranaka, T. (2014). Sterol side chain reductase 2 is a key enzyme in the biosynthesis of cholesterol, the common precursor of toxic steroidal glycoalkaloids in potato. *The Plant Cell*, *26*(9), 3763-3774.

Schaller, H. (2003). The role of sterols in plant growth and development. Progress in lipid research, 42(3), 163-175.

Schrick, K., Mayer, U., Martin, G., Bellini, C., Kuhnt, C., Schmidt, J., & Jürgens, G. (2002). Interactions between sterol biosynthesis genes in embryonic development of Arabidopsis. The Plant Journal, 31(1), 61-73.

Şeker, M., Kemal Gül, M., İpek, M., Toplu, C. L., & Kaleci˙, N. (2008). Screening and comparing tocopherols in the rapeseed (Brassica napus L.) and olive (Olea europaea L.) varieties using high-performance liquid chromatography. International journal of food sciences and nutrition, 59(6), 483-490.

SEVİM, D., KÖSEOĞLU, O., & ÇETİN, Ö. Bazı Önemli Zeytin Çeşitlerinden Elde Edilen Yağların Minör Bileşenlerinin ve Antioksidan Aktivitesinin Belirlenmesi. *Zeytin Bilimi*, *6*(1), 1-7.

Shimada, T. L., Shimada, T., Okazaki, Y., Higashi, Y., Saito, K., Kuwata, K., ... & Ueda, T. (2019). HIGH STEROL ESTER 1 is a key factor in plant sterol homeostasis. *Nature plants*, *5*(11), 1154-1166.

Takemura, M., Tanaka, R., & Misawa, N. (2017). Pathway engineering for the production of β-amyrin and cycloartenol in Escherichia coli—a method to biosynthesize plant-derived triterpene skeletons in E. coli. Applied microbiology and biotechnology, 101(17), 6615-6625.

Tansey, T. R., & Shechter, I. (2001). Squalene synthase: structure and regulation. Progress in nucleic acid research and molecular biology, 65, 157-195.

Tarkowská, D. (2019). Plants are Capable of Synthesizing Animal Steroid Hormones. Molecules, 24(14), 2585.

Umate, P. (2015). Comparison of genes encoding enzymes of sterol biosynthesis from plants to orthologs in yeast. *Rice Research: Open Access*.

Varol, N.Erten, L., ve Turanlı, T. 2009. Zeytin. Tarım ve Köy İşleri Bakanlığı Yayın Dairesi Başkanlığı.Yayın No:52. Ankara

Wang, J., Huang, M., Yang, J., Ma, X., Zheng, S., Deng, S., ... & Zhao, P. (2017). Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. Food & Nutrition Research, 61(1), 1364117.

Yuan, L., Zhang, F., Jia, S., Xie, J., & Shen, M. (2020). Differences between phytosterols with different structures in regulating cholesterol synthesis, transport and metabolism in Caco-2 cells. *Journal of Functional Foods*, *65*, 103715.