# INTEGRATED ALTERNATIVE FARMING MODELS

EDITORS Prof. Dr. Kagan KOKTEN Lecturer Selim OZDEMIR



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## PREFACE

Demand for food resources, which are the most basic in human nutrition and needed for the continuation of life, is increasing day by day. For this reason, the food resources that people need have gained a strategic importance as well as being more vital than many other resources. Hunger arises due to the inability of people to reach quality and sufficient food, and today it is among the most basic problems of societies within the scope of food-security. From the point of view of both easy access to food and agricultural production, the solution is to benefit from technological developments in a sustainable way. These technological developments also have a great potential to contribute to food security.

After the II. World War and since the beginning of the 1950s, the inputs used in agricultural areas caused an increase in productivity, while on the other hand, it also revealed environmental problems. The negative effects of agricultural inputs, which cause environmental problems to reach vital dimensions, have started to be discussed and as a result of the discussions, producers and consumers, especially in developed countries, have started to produce and consume agricultural products that are clean, healthy, do not use chemical inputs, and do not harm people and the environment.

Due to the limited agricultural production areas, the only solution is to increase productivity per unit area. In addition, although the agricultural products produced in our country are gradually increasing and diversifying, their share in the country's economy is gradually decreasing. It has become imperative not to pollute our agricultural lands, to prevent erosion and desertification, to protect our products and to realize sustainable agriculture. There are many weeds, diseases and pests that damage the crops in every period of plant production, which is one of the most important agricultural production activities, from planting to harvest. One of the most important ways to increase productivity in agricultural production is to combat these harmful organisms. The methods of struggle vary depending on the production conditions, the possibilities of the producers and the technology. One of the most preferred control methods to increase productivity in crop production is integrated pest management or, most commonly, integrated control. Integrated combat; Due to its environment-friendly, economic and social responsibility, it is one of the most effective tools of integrated production and integrated product management in agricultural production.

We hope that this book will be useful to all our colleagues working in the field of agriculture. We would like to thank the valuable authors who contributed to the creation of the book and devoted their valuable time to this purpose, and the family of İKSAD Publishing, who made it possible to publish the book.

# Prof. Dr. Kagan KOKTEN Lecturer Selim OZDEMIR

# **CHAPTER 1**

# CHARACTERISTICS OF KAZANKAYA WALNUT VARIETY

Prof. Dr. Ahmet KAZANKAYA<sup>1</sup> Assist. Prof. Dr. Adnan DOĞAN<sup>2</sup> Assist. Prof. Dr. Adnan YAVİÇ<sup>3</sup>

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# **INTRODUCTION**

Turkey is in a transitional position between Asia and Europe due to its geographical structure. Turkey has a high potential in terms of fruit growing due to its current location. Walnut is in an exceptional position among the fruit assets of our country. Compared to other countries, our country has a great wealth in terms of fruit species and varieties (Şen, 2005).

Walnut is systematically included in Class *Dicotyledonae*, Order *Juglandales*, Family *Juglandaceae*, Genus *Juglans* (Şen, 1986). Family *Juglandeceae* includes 60 monoic tree species in 7 genera. The genus Juglans includes 20 species and among these species, the most cultivated species in the world and also valuable in terms of economic return, is "*Juglans regia* L." (Sen, 1986; McGranahan and Leslie 1990, Arzani et al., 2008).

Although Turkey is a rich country in terms of walnut assets, some countries have reached important levels in cultivation as a result of giving great importance to walnut production. Since walnut production in our country is not done with standard grafted walnut varieties, it has not been able to maintain its position in the past.

Walnut is a fruit that is widespread in the world due to its ability to adapt to ecological conditions. Total walnut production is increasing both in the world and in Turkey. As a matter of fact, while the world walnut production was 1.80 million tons in 2005, it was determined that it increased to 3.75 million tons in 2016 (Table 1). In parallel with the walnut production in the world, walnut production in Turkey has also increased, although not at the same rate. Turkey's walnut production, which was 150 thousand tons in 2005, increased to 210 thousand tons in 2017. While the share of Turkey in world walnut production was 8.31% in 2005, it decreased to 5.20% in 2016 (Table 1). According to these data, the increase in walnut production in Turkey is less than the increase in production in the world. Despite this, Turkey is an important walnut producer country and ranks 4th in the world walnut production. Along with Turkey, important walnut producing countries include China, USA, Iran, Mexico, Ukraine, Chile and Uzbekistan. These 8 countries, which have a significant share in walnut production, realize 89.93% of the world's walnut production (Table 2).

	Woi	·ld	Tur	·key	Turkey's Share
Year	Quantity	Variance	Quantity	Variance	in the World
	(Tonnes)	(%)	(Tonnes)	(%)	(%)
2005	1.804.165	100.00	150.000	100.00	8.31
2006	1.854.599	102.80	129.614	86.41	6.99
2007	1.983.722	109.95	172.572	115.05	8.70
2008	2.242.179	124.28	170.897	113.93	7.62
2009	2.475.300	137.20	177.298	118.20	7.16
2010	2.767.609	153.40	178.142	118.76	6.44
2011	3.198.938	177.31	183.240	122.16	5.73
2012	3.660.147	202.87	203.212	135.47	5.55
2013	3.007.937	166.72	212.140	141.43	7.05
2014	3.385.873	187.67	180.807	120.54	5.34
2015	3.589.651	198.96	190.000	126.67	5.29
2016	3.747.549	207.72	195.000	130.00	5.20
2017	-	-	210.000	140.00	-

Table 1. Walnut production in the world and in Turkey

Source: Prepared using FAOSTAT data (Anonymous, 2018a).

Table 2. Important Walnut Producing Countries in th	e World
---	---------

Output	Share (%)	Country	Output	Share (%)
1.785.879	47.65	Ukraine	107.990	2.88
607.814	16.22	Chile	73.529	1.96
405.281	10.81	Uzbekistan	53.116	1.42
195.000	5.20	Total (8 Countrt)	3.370.427	89.93
141.818	3.78	World Total	3.747.549	100.00
	1.785.879 607.814 405.281 195.000 141.818	Output         (%)           1.785.879         47.65           607.814         16.22           405.281         10.81           195.000         5.20           141.818         3.78	Output         Country           1.785.879         47.65         Ukraine           607.814         16.22         Chile           405.281         10.81         Uzbekistan           195.000         5.20         Total (8 Countrt)           141.818         3.78         World Total	OutputCountryOutput1.785.87947.65Ukraine107.990607.81416.22Chile73.529405.28110.81Uzbekistan53.116195.0005.20Total (8 Countrt)3.370.427141.8183.78World Total3.747.549

Source: Calculated from FAOSTAT 2016 data (Anonymous, 2018a)

Walnut production in Turkey is spread over a geographical area close to the whole country. 50-60% of this production is carried out in 20 provinces and Kahramanmaraş, Antalya, Denizli, Bursa and Çorum are the important walnut producing provinces of Turkey (Table 3).

Provinces	Quantity (tonnes)	Share (%)	Provinces	Quantity (tonnes)	Share (%)
Kahramanmaraş	10.902	5.19	Balıkesir	5.085	2.42
Antalya	8.101	3.86	Tokat	5.077	2.42
Denizli	7.962	3.79	Kütahya	4.935	2.35
Bursa	7.409	3.53	Amasya	4.649	2.21
Çorum	6.938	3.30	Hakkâri	4.593	2.19
Mersin	6.452	3.07	Kocaeli	4.528	2.16
Sakarya	6.258	2.98	Manisa	4.520	2.15
Karaman	5.763	2.74	İzmir	4.388	2.09

 Table 3. Walnut production amounts of cities

Van	5.732	2.73	Bitlis	4.071	1.94	
Aydın	5.350	2.55	Total (20 Provinces)	117.825	56.1	
Kastamonu	5.112	2.43	Total (Turkey)	210.000	100.00	
Source: Prepared using TLUK 2017 (Anonymous, 2018b)						

Source: Prepared using TUIK 2017 (Anonymous, 2018b).

Walnut is a very valuable fruit in terms of production and trade. The USA and China are the world leaders in the walnut trade, as they have 1/3 of the world's walnut production areas. The reason for this level of success is that all walnut cultivation is carried out using closed walnut orchards and standard varieties. The important walnut producing countries in EU-27 are Poland, France, Greece and Spain (İmamoğlu, 2015).

When the statistics for 2011 are analyzed in our country, while a total of 183 thousand tons of production was made from an area of 477 thousand decares, the production increased from 718 thousand decares of land to 190 thousand tons in 2015 (Table 4). While the total number of trees was around 9 million 600 thousand in 2011, the total number of trees exceeded 13 million in 2016 (Anonymous, 2016 b).

Year	Collective Orchard Area (decares)	Output (tonnes)	Average Yield per Tree (kg)	Number of Trees at Fruiting Age	Number of Trees at Non- Fruiting Age	Total Number of Trees
2011	468.378	183.240	33	5.594.576	4.045.119	9.639.695
2012	552.019	203.212	34	5.977.397	4.541.958	10.519.355
2013	639.015	212.140	33	6.526.028	4.877.669	11.403.697
2014	693.947	180.807	26	7.000.897	5.374.456	12.375.353
2015	718.196	190.000	25	7.596.020	5.560.227	13.156.247

**Table 4**. Turkey walnut production amount (tonnes) and number of trees (TUIK,2016)

Walnut production varies by regions (Table 5). While the Mediterranean Region takes the first place in production with 17.5%, the Aegean Region is in the second place with 14.9%. These regions are followed by the Western Black Sea and Middle Eastern Anatolia Regions with a ratio of approximately 13.5% from production (Anonymous, 2016b).

	Collective	Outpu	Output(tonnes)%		Total
	Orchard Area (da)	(tonnes)			Number of Trees
Northeast Anatolia	8.663	5.478	2.8	29	259.036
Middle East Anatolia	95.742	25.624	13.5	30	1.309.522
Southeastern Anatolia	37.467	10.028	5.3	23	618.419
Istanbul	2.983	502	0.2	16	76.095
West Marmara	102.413	12.372	6.5	24	1.598.902
Aegean	136.859	28.279	14.9	23	2.241.949
East Marmara	78.117	18.972	10.0	30	1.091.858
West Anatolia	45.902	13.302	7.0	29	781.292
Mediterranean	71.628	33.167	17.5	30	1.571.795
Middle Anatolia	40.093	6.782	3.6	20	737.789
West Blacksea	83.923	25.474	13.4	18	2.237.736
East Blacksea	14.406	10.020	5.3	23	631.854
Turkey Total	718.196	190.000	100	25	13.156.247

**Table 5.** Walnut production amount (tons) and number of trees by regions (TUIK,2016)

All of the cultivars grown in our country have gained the quality of variety with the "selection breeding" method. Some of these varieties are "Yalova 1, Yalova 2, Yalova 3, Yalova 4, Bilecik, Şebin, Gültekin 1, Yavuz 1, Kaplan 86, Şen 1, Şen 2, Tokat 1, Kaplan 86 and also Oğuzlar-77", which has been registered in recent years (Sen et al., 2011).

Walnut production is limited by late spring frosts, mostly by damaging the awakened tissues in the trees. As a matter of fact, the frost event that occurred in 2014 significantly damaged walnut trees in almost all regions of the country. It has been observed that frost damage is effective up to the bole of some trees. This showed how important the selection of late leafing genotypes is in selection studies. In a study started with the aim of determining walnut genotypes that were not affected by the frost that occurred in 2014, superior genotypes were determined in the natural walnut population grown from seed in Adilcevaz district, in accordance with the selection criteria, with late leafing, high yield on the side branches and adapting to the ecology of the region. As a result of the study, considering the selection criteria, the genotype, which has the most superior feature among the genotypes, was registered as the Kazankaya walnut variety and became a standard walnut variety by the Ministry of Agriculture and Forestry. Kazankaya variety is a late leafing variety with high fruit yield on the lateral branches. As a result of previous studies, Kazankaya walnut variety was registered by "Van Yüzüncü Yıl University Nuts Research and Application Center" and included in the standard walnut catalog of our country.

### **Origin of Kazankaya Walnut Variety**

Our country, which is in an important position in terms of fruit growing and culture, is among the homelands of Juglans regia L. In our country, there are walnut populations that are known or defined by the names of their regions in places with rich walnut existence. These are "Adilcevaz, Bitlis, Şebin, Niksar, Kemah, Erzincan, Göynük, Hekimhan, Ermenek, Kaman Walnut" among these examples. The location map of Adilcevaz district, which is the origin center of Kazankaya walnut variety, is presented in Figure 1.

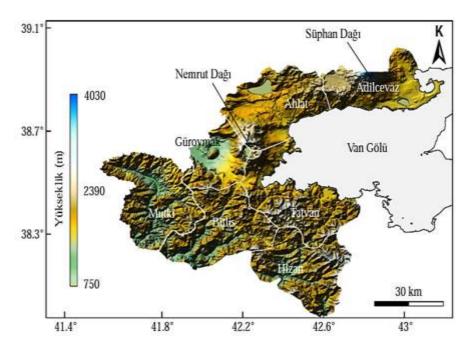


Figure 1. Adilcevaz district location map

### **Characteristics of Kazankaya Walnut Varieties**

Determined characteristics of Kazankaya walnut variety are presented in Table 6, Figure 3.

Juglans regia L

# TEKNIK INCELEME RAPORU

REPORT ON TECHNICAL EXAMINATION

1.	Teknik incelemeyi yapan yetkili kuruluy Testing authority	1	Tohumluk Tescil ve Sertifikasyon Merker Müdüringü ANKARA
2.	Raporu isteyen yetkili kuruluş Requesting authority	I	
3,	Islahçı kişi/kuruluş adı ve adresi Brender (name and adres)	2	Yüzüncü Yıl Üniversitesi Sert Kabuklu Meyveler Araştırma ve Uygulama Merkez Müdürlüğü Tuşba liçesi Kampüşü / VAN
4,	Bayvuru tarihi ve sayısı Date of application in requesting state	1	27.02.2015 / 2993
<u>\$</u> ,	Resource sublishin an execution of residence and adress Applicant (name and adres)	c :	Yüzüncü Yıl Üniversitesi Sert Kabuklu Meyveler Araştırma ve Uygulama Merkez Müdürlüğü Tuşba Bçesi Kampüsü / VAN Prof. Dr. Ahmet KAZANKAYA Yırl. Doğ'Dr. Adnan YAVİÇ Yırd. Doç'Dr. Adnan DOĞAN
6.	Bayvuran temsilci ise ndt ve ndresi Agent (if applicable)näme and adres	- 1	ALLAN .
-	Botanik ndi Botanical name of taxon	-	Andredonics series 1
<u>7.</u>		11	Jeglandaceae regia L
N.	The ads Common name of taxon	10	Ceviz-Valnut
9.	Cepit ada Variety denomination	141	KAZANKAYA
10.	Testing station and place	110	Ciftçi Mehmet AKKOYUN'un bahçesi Oria Mahalle Birinj Caddesi 12765 / 78 Adiloevaz / BITLIS
11.	Test dönemi Period of texting	1	2 311 (2015-2016)
12.	Test sonuçlarının verildiği yer ve tarihi	200 3	Ankara-2017
	Date and place of issue of document		Augurar and
	propagation.) "Y eterince homojen (It sufficiently komogeneous) -Y eterince homojen deĝil. ( is not sufficiently homogeneous)	ve all varie <u>tui</u> dinasi, soni varticulurfe	vies) reu adures of its sexual reproduction or vegutatif
	C) Durulmuşluk rapora (The variety în ite e Çeşit temel baelilikleri, dikkate alınd (According to the basic châracteris -Darulmuş ( is stable)	iginda	100
	-Darulmuş değil ( iz noi stable)		
	-Daratmuş değil ( is not stable) 14. Düşünceler (Remarka) 15. İmza (Signature) Meye ( Fry	t of give	roje Sorumlusu DUS Expert)

Juglam regia L.

#### VARIETY DESCRIPTION Teknik incelemeyi yapan yetkili kurulus **Tohumluk Tescil ve Sertifikasyon Merkez** 1. 1 Testing authority Müdürlüğü- ANKARA 2. Raporu isteyen yetkili kuruluş 1 Requesting authority ADILCEVAZ 2 Cesidin denemelerdeki adı (kod numarası) 3. 1 Variety name or code in tecnical examinatio Yüzüncü Yıl Üniversitesi Sert Kabaklu Başvuru sahibinin adı ve adresi 4. 1 Meyveler Araştırma ve Uygulama Merkez Applicant (name and address) Medarlege Tuşba İlçesi Kampüsü / VAN 5. Islahçı kişi/kuruluş adı ve adresi Yüzüncü Yıl Üniversitesi Sert Kabuklu 2 Meyveler Araştırma ve Uygalama Merkez Breeder (name and address) Müdürlüğü Tuşba İlçesi Kampüsü / VAN Prof. Dr. Ahuset KAZANKAYA Yrd. Dog. Dr. Adnan YAVIÇ Yrd. Doç. Dr. Adnin DOGAN Botanik adı Botanical name of taxon 2 Jugiandaceae regia L. 6. t Ceviz-Falmut 7. Tiir ads Common name of taxon 1 KAZANKAYA Cesit ada Variety denomination 8. Isiah yöntemi Breeding method Seleksiyon 9. 1 10. Ebeveyn adlars Parents line 141 11. UPOV özellik belgesinin tarih ve sayısı 4 TC/125/6 24-03-1999 Date and document number of UPOV test guidelines Test zaman Period of testing 12. Test sonuçlarının verildiği yer ve tarihi Ankara-2017 13. ż Date and place of issue of dociment

CESIT ÖZELLIK BELGESİ

Gözlemlenen özellikler Observationa 14.

UPOV	KAREKTERLER Charocteristics	NOT	ACIKLAMALAR States of expression		
1	Ağaçı Gelişme kuvveti Tree Pigor	5	Orth		
2	Agaç: Bilyilme tablats Tree: Growth habit	1 2	Yayvan Spreading		
3	Ağaç: Dalların yoğunluğu Tree: Density of branches	> 5	Orta		
4	Ağıç: Meyve gözlerinin bulunduğu yer Tree:Predominant location of fruit buds		Geneide 2 ynşında veya daha ya dallar üzerinde uzun sürgünleri Tepesinde (saikum hulinde meyv veren) Mainly on the tap of long shoats bound on branches of 2 years or older firniting in clustersi		
5	Bir yaşlı dal: Renk One-year-old shoot:Color	2	Açık kahverengi Light brown		
6	Yaprak: Yanda bulunan yaprakçık şekli Leaf : Shape of lateral leqflet	7	Genis eliptit		
7	Ciçek: Erkek çiçek sayısı Flawer:Number of male cotkins	5	Orta Aredum 3 3 3		
	Tohumiai Tesci ve Serrifiany Robar Cad. No.(5 PK 20.05) Tel: 0.12 N.1. act. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1.	2 Teninahalla/Ankon			

Tohoniuli Texci ve Serrfikaspor Merkesi Müdürliğü Bağılat Cad. No.62 PK 30-95172 Yenimahaile/Ankara Tel: 0 312 313 46 03 - 315 89 39 Fake: 0 312 315 09 01 Web: www.man.gov.P

Juglans regia L.

5	Meyve: Büyüklüğü Viut: Star	7	Bûyêk Large
9	Meyve: Sarttan uzunlamasına şekli Nut: Shape in longitudinal section through suture	4	Oval Ovale
10	Meyve: Birleşme yerinin uzanlamasına şekli Nut: Shape in longitudinal section perpendicular to zuture	4	Oval Ovate
	Neyve: Birleşme yerinden enine kesit Nut Shape in cross section	3	Eliptik Elliptic
12	Meyve: Yuvarlakhk indeksi Nut: Index of roundness	3	Düşük Low
13	Meyve: Birleşim yerine dik bakıldığında dip kəsmu şekli Nut: Shape of base perpendicular to suture	2	Yuvariak Rownded
14	Meyve: Birleşim yerine dik bakıldığında uç kısmının şekli Nut :Shape of apex perpendicular to sature	1	Sivri Pointed
15	Meyve: Uç kısmın sivrilik şekli Nut : Prominence of apical tip	5	Orta Medium
16	Meyve: Sırttan görünümde dikey yerindeki yastığın uzunluğuna şekli Nut :Position of pad on zuture	1	Üst 2/3'ünde On upper 2/3
17	Meyve: Sirttan görünümde dikey yerindeki yastığın derinliği Nut : Prominence of pad on sature	7	Kuvvetli Strong
18	Meyve: Birleşim yeri gilrünümünde yastığın genişliği Nut : Width of pool on suture	~	Geniş Broad
19	Meyve: Birlesim yeri boyunca oluk derinliği Nut: Depih of grove along pad on anture	5	Orta Medium
20	Meyve: Kabuk yüzeyinin yapısı Nur Structure of surfaçê of shell	4	Kabartinah Embosted
21	Meyve: Kabuk kalinliği Nur : Tihlicknesi of shell	7	Kahn Thick
22	Meyve: Kabuk iki yarısının birleşine derecesi Nut: Adherence of two holves of shell	5	Orta Medium
23	Meyve: leteki primer ve sekonder bölme zarlarının kalınlığı Nut. Thickness of primary and secondary membranes	3	Ince
24	Meyve içi: Çıkarılmı kolaylığı Kernel: Ease of removal	5	Orta
25	Meyve içi: Zemlin renginin yoğunluğu Kernel:Intenzity of ground color	5	Orta
26	Meyve içi: Büyüklüğü Kernel:Size	5	Orta
27	Meyve içi [Randıman]:Kabuklu ağırlık / meyve iç ağırlığını oranı Kernel[Yield] :Percentage of weight relative to total weight of nut	9	Çok Yâksek Very high
28	Olgunlaşma zamanı Time of maturity	6	Orta geç arası Medium to late
19	Yaprak döküm zamanı Tinse of leaf fall	2	Geç Late
30	Yaprak: Sap uzunluğu Leaf:Persistence of rachis	1	Sürekli değil Not persistent
31	Yeşil kabuk: Ağaçtan döküldöğünde sert kabuktan ayrılmaya dayanıklılığı Hask:Persistence on iree ufter nut fall	1	Kalıcı değil Not peraintent
32	Yaprak tomurcuğunun patlama zamanı Time of leaf bud burst	6	Orta geç araşı Medium to late
33	Erkek cicek açma zamanı Time of male flowering	4	Orta erken

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Juglans regia L.

34	Dişi çiçek nema zamanı Time of female flowering	5	Orta Medium
35	Erkek çiçehlerin dişi çiçeklere göre çiçek açına zaman Time of male flowering compared ta female flowering	1	Önce Before (protandry)

# 11.Benzer çeşitler ve bu çeşitlerden farklıkları (\*\*\*) Similar varietiez and differencez from these varietiez

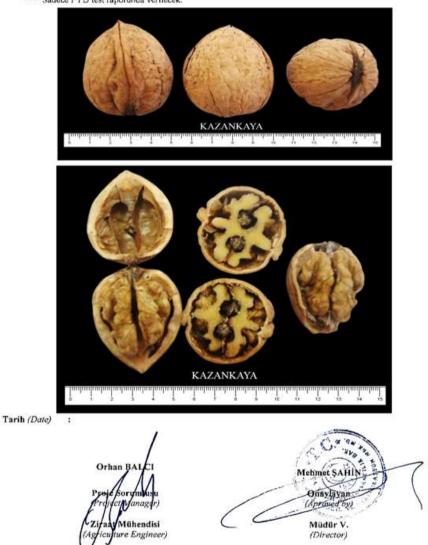
Benzer çeşidin adı Denominotion of similar variety	Benzer çeşitten farklı Xarakterleri <sup>(**)</sup> Characteristics in which similar variaty is different	Benzer çeşitte karakterin görünümü State of expression of similar variaty		Aday çeşitte karakterin görünümü State of expression of candidate variety	
Adilcevaz I	L.Buyûme şekli Tree: Growth habit	3	Yayvan Spreading	2	Yarı dik Semi-upright
	7. Çiçek: Erkek çiçek şaym Flower:Number of müle catkins	5	Orta	3	Bir kaç Few
	8. Meyve: Büyüklüğü Nut :Size	7	Bayah Large	5	Orta Medium
	9. Meyve: Seritan uzunlamasna bicleşme yeri şekli Nut:Shapa in longitudinal section through suture	-/	Oval	2	Daire Circular
/	10. Meyve: Birleyne yerinin uzunlamasına yekli Nut :Shape in longitudinal section perpendicular to suture		Oval Ovare	3	Daire Circular
1:	11. Meyve: Birleyme yerinden enine kesiti Nut "Shape in cross section	K	Eliptik	2	Dairesel Circular
~	12. Meyve: Yuvarlakisk indeksi Nut (Index of roundness	3	Düşük	5	Orta Medium
N. N. N.	13. Meyve: Birleşim yerine dik bakıldığında dip kısının yekli Nur Shape of base perpendicular to suture	2	Yuvarlak Rounded	3	Kút Truncate
	14. Meyve: Birleyin yerine dik bakıldığında uç kusunan şekli Nur. Shape of opex perpendicular to suture	1	Sivn Pointed	1	Rounded
	19, Meyve: Birlepint yeri boyunca oluk derinligi Nut: Depth of grove along pad on suture	2	Orta Medium	3	Y lizeysel Shallow
	23. Meyve: leteki primer ve vekonder bölme zariarının kalınlığı Nut :Thickness of primary and secondary membranes	81	Thin	5	Orta Medium
	28. Olgonlaşına zamanı Time of maturity	6	Orta-geç arası Medium to late	5	Orta Medium
	32. Yaprak tomurcuğunun patlama zamunı Time of leaf bud burst	5	Orta Medium	6	Orta-geç arası Medium to late
	33. Erkek cicek acma zamani Time of male flowering	5	Orta Medium	6	Orta-gaç arası Médium to lole.
	34. Disi cicek acma zamanı Time of female flowering	5	Orta Medium	6	<ul> <li>Orta-gettariisi</li> <li>Medium To fbge</li> </ul>

(S)

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Juglans regia L.

\*\* Benzer çeşitler yazıldığı takdirde; bu çeşitlerden farklılıklarını gösteriniz. (İn the case of identical state of expressions of both varieties, please indicate the size of difference) \*\*\* Sadece FYD test raporunda verilecek.



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Figure 3. Characteristics of Kazankaya variety.

Comparison of some standard walnut varieties grown in Turkey and in the world in terms of some quality characteristics of Kazankaya Walnut variety is presented in Table 6.

Variety	Shell Fruit Weight (g)	Internal Weight (g)	Internal Rate (%)	Flowering Feature
Kazankaya	15.11±1.21	7.86±0.63	52-60	Protandry
Yalova- 2	16.50	7.60	46.10	Protogyny
Yalova- 3	12.00	6.40	53.30	Protandry
Bilecik	12.76	6.51	50.00	Protandry
Şebin	10.00	6.60	63.00	Protogyny
KR- 1	10.90	7.20	66.00	Protogyny
60- TU- 1	12.00	7.60	63.30	Protandry
Maraş- 12	8.02	5.36	67.00	Homogamy
Maraş- 18	14.84	7.98	53.77	Protogyny
KSÜ- 14	12.48	7.36	59.04	Protandry
Vina	12.85	6.30	49.02	Protandry
Franquette	10.75	4.78	44.46	Protandry
Mayette	10.50	4.57	43.52	Protandry
Tulare	13.30	7.10	53.38	Protandry
Payne	11.40	5.70	50.00	Protandry
Amigo	11.57	5.90	51.00	Protogyny
Serr	13.68	7.80	57.01	Protandry
Champion	14.00	8.12	58.00	Protandry
Chandler	13.26	6.50	49.02	Protandry
Fernor	13.00	6.76	52.00	Homogamy
Fernette	11.30	5.54	49.00	Homogamy

Table 6. Characteristics of Standard Walnut Varieties and Kazankaya variety

Based on the Shell Fruit Weight (g), Internal Weight (g) and Internal Ratio (%) characteristics, it is observed that Kazankaya variety is superior to foreign standard walnut varieties and domestic walnut varieties specified in the table which are Yalova-3, Bilecik, Şebin, KR-1.60-TU- 1, Maraş-12, Maraş-18 and KSÜ-14 (Şen et al., 2006)

Another superior feature is that it is a walnut variety that should be preferred in high altitude regions, considering its late leafing and resistance to both high altitude and vegetation due to the fact that it has an altitude of 1700 meters in terms of the region where it is selected.

# CONCLUSION

It has been determined that Kazankaya walnut variety has many superior features when compared with other varieties. In addition, it is expected that significant benefits will be provided if the production of this variety becomes widespread in the region. Kazankaya walnut variety is a variety selected from the Adilcevaz region at an altitude of 1700. Considering the climate, altitude and other limited factors, the fact that it is more durable than other varieties and its late foliation reveals its importance.

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# CHAPTER 2

# COVER SOWING METHOD IN TURF GRASS

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# **INTRODUCTION**

Grass fields are an important element in human social life. Grasses are not only pleasing to the eye with their green appearance in recreation areas, but are also preferred for resting needs and sportive activities. In busy city life, grass reduces sound pollution by absorbing and softens the microclimate by reducing the temperature of the environment in summer.

The ideal turf field establishment is very difficult, but it is a work that requires care. It is very important that the grass is emerald green in color and homogeneous with less weed content. If this is not achieved, large gaps are formed between the grass, making the area unusable and weeds may become dominant. By causing these problems; It can be shown that the settled soil material is not processed well, bad leveling, weed parts remain alive under the soil, and non-experts carry out the work.

A patent application has been made for the cover planting method; with this method, less seeds are used in grass field sowing. An important effect is its success in weed control. The difference of the sowing method is revealed by using fiber cover in the processing stages of this method. Commonly used sowing methods are; classic grass sowing, roll grass sowing, hydroseeding grass sowing.

# 1. COARSE TILLAGE AND ORGANIC FERTILIZATION

In the grass field plant, the main soil material is brought from different areas. Generally, it is desired that the ideal soil material is cultivated field soil. In cases where field soil is not available, excavation soils are preferred as an alternative. However, since these soils are poor in terms of nutrient content, they should be enriched.

One of the most widely used methods while enriching the soil nutrient content is the use of organic fertilizers. Care should be taken that this food source is fermented. Unfermented (unburned) fertilizers do more harm than good to the soil. The roots of newly germinating plants may burn to death due to fertilizer. This error is quite common in general agricultural practices.

Table 1. Annual organic Fertilizers And The Annount To be Osed		
Organic Fertilizers	ton/da	
Cattle Manure	5	
Sheep Manure	4	
Poultry Manure	3	
Sewage Sludge	2	

Table 1: Animal Organic Fertilizers And The Amount To Be Used

Organic fertilizers should be mixed with the main soil piles before rough leveling (Table 1). In particular, the use of construction equipment facilitates this process and contributes to a more homogeneous mixture. Fermented organic fertilizers not only enrich the soil in terms of nutrients, but also improve its physical structure and increase its water holding capacity.

It is also possible that there will be vegetable soil on the ground in the area where the grass plant will be built. Soil; It should be ventilated by turning it upside down with plows and similar equipment and free of live weed parts. After the rough leveling of the transported or processed soil material is done, the fine leveling phase is started.

# 2. FINE TILLAGE AND INORGANIC FERTILIZATION

While the soil is subjected to final processing with the harrow, inorganic fertilization is also done at the same time. While granular fertilizer containing 6-8 kg/da of pure P is applied to the soil only once during planting, granular fertilizer containing 5 kg/da of pure N is applied to the soil every month during the vegetation period and during the development of the grass plant after sowing (Table 2). The fertilizer to be applied is mixed into the soil 3-5 cm deep with the harrowing method. This process should be applied homogeneously to the area by experienced people.

Inorganic Fertilizers	kg/da/month (pure N)
Ammonium sulfate	24
Ammonium nitrate	15
Urea	10
DAP	14 + other nitrogen fertilizer supplements (sowing time)
Other comp. fertilizers	Recommended according to N ratios (sowing time)

Table 2: Fertilizer Doses To Be Applied

Immediately after harrowing and fertilizing, the soil should be pressed well by applying a roller. The excessive amount of air in the uncompressed soil causes the death of the plants after germination.

# 3. LAYING AGRIL COVER

Multiplex polypropylene fiber cover has a weight of  $19 \text{ g/m}^2$  and has a dense texture. Because it is an organic material, it breaks down and mixes with the soil in a few years. It does not harm the soil, plant or environment like chemical wastes. Because the cover is very thin and light, it can be easily transported to the grass plant area where the application will be made.

After fine tillage, a cover is laid over the entire area where the grass will be sown. This enables the seeds to be level after sowing and prevents them going further deeper into the soil. Since the seeds are prevented from going further deeper into the soil, the emergence problems are eliminated. Thus, the amount of seeds to be planted per  $m^2$  is reduced enabling seed saving.

The most beneficial aspect of the cover is to prevent the emergence of weeds from the bottom. Leaf sheaths of weeds cannot pierce the cover and cannot rise to the soil surface. However, it allows the passage of the plant roots germinating on it and does not prevent its formation in the lower layers. Compared to other sowing methods, cover enables the germinating plants look more homogeneous and are completely free of weeds.

Grass fields established with the cover sowing method can also be harvested as roll grass. As a matter of fact, roll grass cutting of the plant established with this method was made in the campus of Iğdır University. The transferred grass rolls continued their vital development in the area where they were laid.

# 4. COVER SOIL AND SEED SOWING OPERATIONS

The cover soil mixed with the seed is laid over the cover that is above the ground. The inorganic fertilization process applied in fine tillage can also be transferred here. In this case, the cover is made from a mixture of soil, seeds and inorganic fertilizers. Especially sowing time should be done in mild climate periods such as spring and autumn. It is known that sowing works carried out during the summer periods are generally unsuccessful due to extreme temperatures.

Cover Soil: The most beneficial and ideal is the use of only peat material. However, in cases where economic conditions do not allow due to the cost of the peat material, semi-sieved field soil can also be used. In this method, the sifted soil should be mixed with the peat to be homogeneous. While the mixing process is done with heavy machinery for large areas, it is done by using human labor with the help of shovels for small areas. However, while preparing the mixture, care should be taken not to mix sand and animal manure into the materials. Sand particles reflecting sunlight cause the death of newly germinated plants by burning the leaf sheaths. Animal manure, on the other hand, poses a risk in the establishment of lawn fields, as it may contain weed seeds.

Seed: *L. perenne*, *P. pratensis*, *F. arundinacea*, *F. rubra rubra*, *F. rubra comutata*, *F. rubra trichophylla*, *A. tenuis*, *A. stolonifera*, *F. ovina* species and varieties from cool climate grass types are used in the establishment of grass fields. *C. dactylon* and *Zoysia* sp. species and varieties are used from hot climate grass species. The determination of grass seed rates varies according to the climatic conditions of the environment and the purpose of use. Generally, mixtures of 3 to 9 varieties are prepared (Table 3). While 40-50 gr/m<sup>2</sup> seeds are used in the normal grass field facility, 30-35 gr/m<sup>2</sup> seeds are used in cover grass field facility.

	Mixing ratios (g/m <sup>2</sup> )				
Species	Sample 1	Sample 2	Sample 3	Sample 4	
L. perenne	14	6	-	-	
F. rubra rubra	7	5	4	-	
F. rubra comutata	7	5	-	-	
F. rubra trichophylla	-	5	5	-	
F. arundinacea	-	8	-	-	
P. pratensis	5	4	5	-	
A. tenuis	-	-	3	-	
C. dactylon	-	-	-	20	

Table 3: Sample Species Mixtures That Can Be Used In The Cover Sowing Method

After the preparations are made, the calibration work is done first. The amount of cover soil (1 cm thick) per unit area is determined. Then cover the soil and seeds are mixed thoroughly. The mixture prepared with a hand shovel is sprinkled on the floor covering. After that, the well compaction of soil is achieved by cylinder passages.

# 5. WATERING AND MOWING

After sowing, the area should be watered regularly. In mild climate periods, irrigation once a day will be sufficient. Irrigation of the area should be done at sunrise in the morning or close to sunset in the evening. Irrigation should be done with a pre-installed sprinkler irrigation system. In cases where there is no irrigation system, sprinkler irrigation can be done with a hose. The factor that determines the irrigation frequency is the ambient temperature, and the frequency and amount of irrigation can be increased or decreased depending on the weather conditions in the following time periods. Care should be taken that the germinating plants are mowed from the top and that the roots do not move during the mowing. For this reason, passing over the grass area with a roller immediately after the mowing ensures the strengthening of the root-soil contact. As the plants develop, care should be taken to mow before laying occurs. It will be seen that within about three months after sowing, the grass is structured and covers the whole ground.

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# **CHAPTER 3**

# ROOT ROT PATHOGEN IN PISTACHIO (Pistacia Vera L.):

# Rhizoctonia solani

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# **INTRODUCTION**

Pistachio (*Pistacia vera* L.) is a thin-shelled, oily, edible fruit and belongs to the family Anacardiaceae. It is mostly used in dessert making and various food industries. The homeland of the pistachio tree is the Middle East and Central Asia. Archaeological evidence indicates that pistachios were consumed around 6750 BC. The form of pistachio grown today is of Bronze Age origin and its lineage is based on the geography of Uzbekistan (Anonymous, 2021). It is stated that pistachios have positive effects on the body by reducing the cholesterol level in human blood, reducing the risk of coronary heart disease, and preventing the rise of blood sugar (Tunalioğlu ve Taşkaya 2003; Razavi, 2010).

Pistachio yields are high in one year, while the yield decreases the next year depending on the periodicity. Despite this, production has been increasing over the years. When the average values (tons) of pistachio production are compared, the highest production is in the USA, Iran, Turkey, China, Syria and Greece, respectively. (Anonymous 2018).

One of the important factors limiting the production of pistachio is diseases and pests. The main pests are; Agonoscena *pistaciae* Burck. and Laut., *Kermania Pistacella* Ams., *Thaumetoopoea Solitaria* Frey. *Hylesinus Vestitus* M.R., *Capnodis spp.* The important diseases seen in pistachios are given in the table below.

Disease name	Disaese factor
Alternaria late blight	Alternaria alternata (Fr.) Keissl.
Aspergillus fruit rot	Aspergillus niger Tiegh.
Armillaria root rot	Armillaria mellea (Vahl:Fr.) P. Kumm
Flower and shoot blight	<i>Botrytis cinera Pers.:Fr.</i> (Telemorph. <i>Botryotinia fuckeliana</i> (de Bary) Whetzel.)
Cotton root rot	Phymatotrichopsis omnivora (Duggar) Hennebert
Phtytoptora crown rot	Phytophtora capsici P. palmivorum P. citrophthora Phytophtora sp.
Eutypa backwards death	<i>Eutypa lata</i> (Pers.:Fr.) Tul. And C. Tul.(anamorph. Libertella blepharis A. L. Smith )
Gum cancer	Cytospora terebinthi Bres.
Leaf spot	Phyllosticta lentisci (Pass.) Allesch.
Panicle and Shoot	Botryosphaeria dothidea (Moug.:Fr.) Ces. and De Not

Table 1. Diseases of Pistachio (Teviotdale et al., 2001).

Blight	
Phomopsis blight	Phomopsis sp.
powdery mildew	Phyllactinia angulata (E. S. Salmon) S. Blumer
Rust	Pileolaria terebinthi Castagne=Uromyces terebinthi (DC.) G. Wint.
Sclerotinia shoot blight Seedling blight Septoria leaf spot disease	Scelerotinia sclerotiorum (Lib.) de Bary Rhizoctonia solani Kühn (telemorph Thanatephorus cucumeris (A. B. Frank ) Donk)Septoria pistacina Allescher (telemorph Mycosphaerella pistacina Chitzanidis)Septoria pistaciarum Caracc. (telemorph
	Mycospharella pistaciarum Chitzanidis)
Verticillium wilt	Verticillium dahliae Kleb.
Fusarium root rot	Fusarium solani
and wilt	Fusarium proliferatum
	Fusarium oxysporum
	Fusarium redolens
Rhizoctonia root rot	Rhizoctonia solani

The most important of these diseases are; Septoria leaf spot (*Septoria pistacina ,Septoria pistaciarum*, Verticillium wilt (*Verticillium dahliae*), Panicle and Shoot Blight (*Botryosphaeria sp.*), Phtytoptora crown rot (*Phytophtora spp.*,) Alternaria late blight (*Alternaria spp.*,) and Armillaria root rot (*Armillaria mellea*).(Anonymous, 1997). Among these pathogens, it has been stated that root rot caused by *R. solani* is especially important in seedlings (Aydın and Ünal, 2021).

#### Rhizoctonia solani as the pathogen

Rhizoctonia solani Kühn, a soil-borne fungus [Telemorph: Thaatephorus cucumeris (FR) Donk] causes disease in many plants and can survive in the soil for a long time due to its specific sclerotic structures (Boosalis and Scharen, 1959). The pathogen, which generally causes various diseases such as root and crown root rot, leaf and stem burn, and can be found everywhere of the plants (Clarkson and Cook, 1983; Carling et al., 1994; Mohammadi et al., 2003). The main hosts are annual plants such as barley, pepper, wheat, tomato, bean, carrot, clove, cauliflower, chickpea, potato, sugar beet, soybean, tobacco, alfalfa, and also many fruits and forest trees such as pistachio. The life cycle of *Rhizoctonia solani* is given in figure 1.

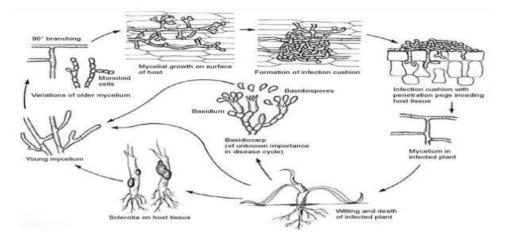


Figure 1. The life cycle of Rhizoctonia solani

According to the circle of life, *Rhizoctonia solani* has the ability to form sclerots on plant remains and in soil. These sclerotia germinate to form hyphae, which cause infection in plant tissues. In addition, basidiums and basidiospores are formed as a result of sexual reproduction in these mycelial layers. The fungus is a soil and seedborne pathogen and can survive as mycelium in plant tissues that decompose in the soil. The inoculum of the pathogen shows a significant reduction in the absence of host plants. In addition, soil type, rotation and the amount of organic matter in the soil also affect the reduction of the pathogen. Suitable conditions for disease development are cool weather conditions (18-22 °C) and moist soils. Red-brown spots and canker sores occur on the stems and roots of diseased plants.

The relationship between *Rhizoctonia* species are characterized by hyphal fusion conditions (Ogoshi, 1987). The groups having hyphae fuse with each other are called "anastomosis group (AG)". Currently, fourteen (AG 1-13 and BI) anastomosis groups of *R. solani* and eighteen anastomosis groups of binucleate *Rhizoctonia* have been identified (Sneh et al., 1998; Yang et al., 2015; Dong et al., 2017). Figure 2 shows anastomosis between hyphae (a) and fungal culture grown in PDA medium(b).



(a) (b) Figure 2. Confluence of *Rhizoctania Solani* hyphae (a), image of colonies growing on PDA medium(b).

The hosts of the pathogen; potatoes, tomatoes, beans, cucurbits (such as cucumber, watermelon, pumpkin and melon), sugar beet, peanut, clover, lettuce, eggplant, corn, strawberry and various fruit trees. In peanuts, it is seen especially in the sapling period or in the first years after planting in the field.

There are many races or pathotypes of Rhizoctonia fungus and they are called anastomosis groups. Different groups of anastomosis cause disease in different hosts. For example, it has been reported that the strain causing disease in potatoes is AG-3 (Aydın and Bağış, 2019). it has been reported in Iran that R. solani AG4 causes wilt, root and root rot diseases in many pistachio saplings (Ashkan and Abusaidi, 1995; İlkhan et al., 2011). In another study conducted by Aydın and Ünal (2021), in Siirt province in the Southeastern Anatolia Region of Turkey, the pathogenicity and anastomosis groups of seven Rhizoctonia-like isolates obtained from diseased pistachio seedlings were determined. In the pathogenicity test, the most virulent isolate was Rs2 with 93.75% disease severity. It was determined that this isolate caused the seedlings to dry completely and Rhizoctonia solani AG-4 in the anastomosis group. The moderately virulent Rs3 and Rs1 isolates were determined as the binucleate AG-F group. These two isolates caused vellowing of the seedling leaves and browning of the roots, however the plants did not die and preserved their vitality to a certain extent. The R9 and R14 isolates identified as anastomosis group AG-F did not cause any symptoms on inoculated plants and on the negative control. The Rs7 and Rs10 isolates were identified as the anastomosis group AG-4 and were determined to be pathogenic in saplings. The results of this study are the first record for

the virulence and anastomosis groups of *Rhizoctonia* species that cause root and crown root diseases in Siirt pistachio. Figure 3 shows images from this study. It was observed that the leaves of the pistachio sapling infected with *Rhizoctonia solani* AG-4 turned yellow and started to fall. After this plant was removed, the root and root collar parts were examined and it was stated that there were signs of decay and browning in those parts and reductions in the fibrous roots. it has been reported that *R.solani* is important among soil pathogens that cause root rot and wilt in pistachio in other studies conducted in the Siirt region of Turkey (Aydın, 2019a; 2019b).



**Figure 3.** Wilting symptoms of *R.solani* Rs2 isolate in pistachio seedlings (a), and darkening of the roots(b)

#### Disease control in pistachio caused by R. solani

There is no effective and economical chemical control against the pathogen. The following points should be considered in the control against the disease caused by *R. solani* in pistachio

The saplings planted in the orchard should be disease-free and healthy. Plants should not be injured. Planting of saplings should be done in warm and less humid conditions. Soil drainage should be given importance in nurseries and orchard and water accumulation should not be allowed. Saplings should not be planted close to each other and providing ventilation. Compost and organic fertilization should be done carefully, and excessive fertilizer not given to the root zone. The disease is less common in sandy soils, but more common in heavy soils. Therefore, attention should be paid to the soil structure of the production areas. Diseased plants should be removed from their growing area and destroyed.

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### **CHAPTER 4**

### **ROOT ROT AND WILT PATHOGEN IN PISTACHIO (Pistacia** Vera L.): Fusarium spp.

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#### **INTRODUCTION**

Pistachio (*Pistacia vera* L.) is a thin-shelled, oily, edible fruit and belongs to the family Anacardiaceae. It is mostly used in dessert making, pharmacy and various food industries(Anonymous, 2013).. Pistachio has many positive effects on health; It is a protein and mineral store, and it is stated that the risk of heart attack and cancer decreases thanks to the calcium, phosphorus, B1, B2, C and E vitamins it contains. In addition, it is reported that it strengthens the mind, gives energy to relieve fatigue, is good for the respiratory and digestive system, and has an anti-inflammatory effect (Çınar ve Okay, 2013). The homeland of the pistachio tree is the Middle East and Central Asia. Most production in the world is made in the USA, Iran, China and Turkey (Anonymous, 2013). Although pistachio is grown in 56 provinces in Turkey, more than 90% of the production is in the Southeastern Anatolia Region. This region has pioneered the successful cultivation and spread of pistachios, as it is the gene center and the place where it was first cultivated, and due to its ecological characteristics. (Yavuz ve ark. 2016).

There are several factors that limit pistachio production. These include losses caused by environmental-physiological factors, lack of fertilization, irrigation problem, variety selection, periodicity and disease-pests. One of the important factors limiting the production of pistachio is diseases and pests. The main pests are; *Agonoscena pistaciae* Burck. and Laut., *Kermania Pistacella* Ams., *Thaumetoopoea Solitaria* Frey. *Hylesinus Vestitus* M.R., *Capnodis spp.* (Anonymous,2008). The important diseases seen in pistachios are given in the table below.

Disease name	Disease factor
Alternaria late blight	Alternaria alternata (Fr.) Keissl.
Aspergillus fruit rot	Aspergillus niger Tiegh.
Armillaria root rot	Armillaria mellea (Vahl:Fr.) P. Kumm
Flower and shoot	Botrytis cinera Pers.: Fr. (Telemorph. Botryotinia fuckeliana
blight	(de Bary) Whetzel.)
Cotton root rot	Phymatotrichopsis omnivora (Duggar) Hennebert
Phtytoptora crown	Phytophtora capsici
rot	P. palmivorum
	P. citrophthora
	Phytophtora sp.
Eutypa backwards	Eutypa lata (Pers.:Fr.) Tul. And C. Tul.(anamorph. Libertella
death	blepharis A. L. Smith )

**Table 1.** Diseases of Pistachio (Teviotdale et al., 2001).

Gum cancer	Cytospora terebinthi Bres.
Leaf spot	Phyllosticta lentisci (Pass.) Allesch.
Panicle and Shoot Blight	Botryosphaeria dothidea (Moug.:Fr.) Ces. and De Not
Phomopsis blight	Phomopsis sp.
powdery mildew	Phyllactinia angulata (E. S. Salmon) S. Blumer
Rust	Pileolaria terebinthi Castagne=Uromyces terebinthi (DC.) G. Wint.
Sclerotinia shoot blight Seedling blight	Scelerotinia sclerotiorum (Lib.) de Bary Rhizoctonia solani Kühn (telemorph. Thanatephorus cucumeris (A. B. Frank ) Donk)
Septoria leaf spot disease	Septoria pistacina Allescher (telemorph. Mycosphaerella pistacina Chitzanidis)
	Septoria pistaciarum Caracc. (telemorph. Mycospharella pistaciarum Chitzanidis)
Verticillium wilt	Verticillium dahliae Kleb.
Fusarium root rot	Fusarium solani
and wilt	Fusarium proliferatum
	Fusarium oxysporum
	Fusarium redolens
Rhizoctonia root rot	Rhizoctonia solani

The most important of these diseases are; Septoria leaf spot (*Septoria pistacina ,Septoria pistaciarum*, Verticillium wilt (*Verticillium dahliae*), Panicle and Shoot Blight (*Botryosphaeria sp.*), Phtytoptora crown rot (*Phytophtora spp.*,) Alternaria late blight (*Alternaria spp.*,) and Armillaria root rot (*Armillaria mellea*). Among these pathogens, it has been stated that root rot caused by *Fusarium spp.* is especially important in sapling (Nouri et al 2018; Aydın, 2019a).

#### Fusarium spp as the pathogen

The mycelium of the fungus is segmented and often irregularly branched. Macro and microconidia are formed on the conidia carriers. Macroconidia are sickle-shaped with 3-6 compartments depending on the species. microconidia are unicellular and colorless. The fungi overwinter in the soil and plant residues in the form of mycelium, condiospores or chlamydiaspores (Barnett and Hunter, 1998; Burgess et al.,1998). *Fusarium* species usually cause seed, seedling rot, root and crown rot and wilt symptoms in plants. As a result of the clogging of the transmission bundles in plants, wilting and drying occur in the advanced stage (Arie, 2019). The pathogen causes disease in many plants. Plants such as wheat, corn, barley beans, cucurbits, strawberries, anise are among its hosts. In addition, Fusarium species cause damage to many forest plants as well as cultivated fruit trees, including pistachios.

#### Fusarium spp. in pistachio

Disease symptoms begin with yellowing and wilting and especially in hot July-August months, it is seen as sudden drying. When the root collar of the plants showing signs of disease is cut transversely, discoloration of the vascular bundles can be seen. The signs of wilting and drying are seen in different periods. These symptoms can be seen suddenly within 3-4 years after planting, or they can be seen after the age of 4-5 after the stress conditions caused by some cultural processes on the plant. There is a relationship between plant age and drying, and wilting and drying are more common at early ages. The disease can be seen in almost all areas where pistachios are grown. Significant economic losses are experienced due to the uprooted plants after drying. Disease damage is tried to be compensated by planting new saplings in the orchard(Anonymous,2018)

The main *Fusarium* species that cause disease in pistachio; *Fusarium* solani, *F. proliferatum*, *F. equiseti*, *F. oxysporum* and F. redolens, *F. brachygibbosum*, *F. chlamydosporum* (Nouri et al 2018; Aydın, 2019a; Crespo Palomo et al.,2019; Demiray ve Akçalı, 2020). Some *Fusarium* species and their characteristics that are important for pistachios are given below.

#### F. solani (Telemorf: Nectria haematococca)

F. solani has aerial hyphae that give rise to conidiophores laterally. The conidiophores branch into thin, elongated monophialides that produce conidia Phialides that produce macroconidia are shorter than those that produce microconidia The macroconidia produced by F. solani are slightly curved, hyaline, and broad, often aggregating in fascicles. Typically the macroconidia of this species have 3 septa but may have as many as 4-5. forms chlamydospores most Fusarium *solani* also commonly under suboptimal growth conditions. These may be produced in pairs or individually. They are abundant, have rough walls, and are 6-11 µm. F. solani chlamydospores are also brown and round (Summerel et al., 2010). The images of micro-macro conidia and phyalids of F.solani under the microscope are given in figure 1.



Figure 1. The images of micro-macro conidia and phyalids of F.solani under the microscope

## *Fusarium proliferatum* (Matsushima) Nirenberg (Telemorf: *Gibberella intermedia* )

Their macroconidia are cylindrical, flat, and usually 3-5divided. On the other hand, microconidia are usually formed on polyfialids and rarely monofialids in chain form. Microconidia are pear-shaped with the basal part flat. Does not have chlamidospores (Anonymous, 2022). The images of micro-macro conidia and phyalids of *F. proliferatum* under the microscope are given in figure 2.

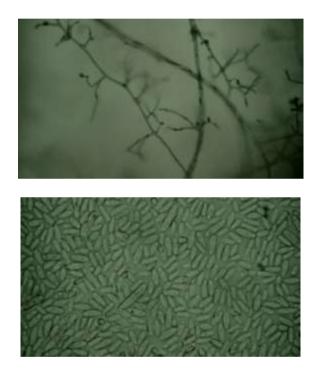
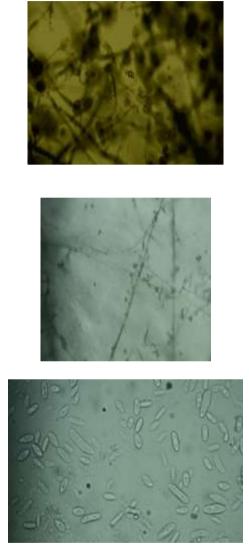


Figure 2. The images of micro-macro conidia and phyalids of *F. proliferatum* under the microscope

### *Fusarium oxysporum* (Schlecht as emended by Snyder and Hansen)

Conidiophores are short, solitary, lateral monofialids arranged in densely branched clusters Under the microscope, Chlamidospores are transparent, smooth or rough-walled, singly or in pairs at the end or intermediate part of the hyphae.Macroconidia are curved, slightly curved, pointed at the apex, and mostly 3-5-septate. Microconidia are oval, elliptical, or kidney-shaped, undivided or one-septate, straight or often curved (Leslie, and Summerell, 2006). The images of micro-macro conidia and phyalids of *F*. *oxysporum* under the microscope are given in figure 3.



**Figure 3.** The images of chlamydospore, micro-macro conidia and phyalids of *F*. *oxysporum* under the microscope

# *Fusarium equiseti* (Corda) Sacc. (Telemorf: *Gibberella wollenw*)

Macroconidia, usually 5 to 6 septate, 31 to 45  $\mu$ m long, have a prominent dorsiventral curvature, tapering and elongated apical cell, and prominent foot. Microconidia should have two to three septate. Chlamidospores are produced as spherical (7 to 13  $\mu$ m) hyphae, often forming chains or clusters. (Hami et al.,2021).

Important studies on the diseases caused by Fusarium species in pistachios are given below. In a study conducted in Syria in 2014-2015, a disease that caused the death of 50-60% of sapling in pistachio nurseries was detected. Lesions showing reddish brown and black coloration were detected on the roots of diseased sapling. As a result of isolation from infected tissues, it has been reported that the factor causing this disease is *F.solani* (Walid ve Abeer, 2017).

Some Fusarium species have been reported to infect plants in pistachio orchards, especially in nurseries (Trika et al., 2011; Nouri et al.,2018 Cerespo et al.,2019). The most characteristic symptom on the plant is rot in the roots and root collar. In these decaying areas, reddish brown or blackish images occur. In the last stage, wilting and drying occur in the saplings. It was reported that the drying of pistachio nurseries was caused by Fusarium solani in the studies conducted in Tunisia in 2011 and in Syria between 2014 and 2015. In addition, this pathogen has been reported as the first record in pistachio for the mentioned countries (Triki et al., 2011; Walid and Abeer, 2017). In addition to some pathogens, Fusarium oxysporum, F. solani, F. equiseti and F. proliferatum species were also isolated in studies conducted to determine diseases in pistachio trees in California (Nouri et al., 2018). It has been determined that these fungi cause discoloration and wilting in the vascular bundles of plants. The symptoms caused by Fusarium species in pistachio saplings and trees are given in figure 4.



Figure 4. The symptoms caused by Fusarium species in pistachio saplings and trees

#### Control of root rot and wilt disease caused by Fusarium spp.

Orchard planting should be done with certified seedlings. The pits opened in planting should be well filled and deep sapling should not be planted. Cultivation should not be done with plants sensitive to *Fusarium* species (pepper, melon, watermelon etc.). It should be done in accordance with the grafting technique, and the plant should not be stressed by excessive branch cutting after grafting. Plants that dry up after the disease should be destroyed after they are removed and quicklime should be filled into the removal pits. It should be done in accordance with the irrigation technique and care should be taken not to let the water touch the stems of the saplings/trees during irrigation. There is no effective chemical control method against this disease.

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### CHAPTER 5

#### AGRICULTURAL WATER STRUCTURES

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#### **INTRODUCTION**

Water structures are widely used in cities and villages to transmit and store water, to prevent floods and to generate energy. Protection of the fruitvegetable lands and livestock shelter from floods is very important in terms of agriculture. Since floods cause great economic damage to agriculture sector. Therefore, water structures should be built in agricultural areas. Besides, these structures are also beneficial as they store water and transmit it to the lands. Some water structures in villages are cement pipe, culvert, crossdrain, dam and bridge. This study aims to develop a perspective on water structures in terms of agriculture. Because the water structures are very useful in water storage for irrigation and they prevent the flood.

#### Floods

While the torrent was sudden in the tributary river and upper basins, floods occur in the valley plain and downstream basins. Torrent carry high sediment while flood water carries low sediment. Although the main reason for the flood is precipitation, the intervention of people in the land cover and the constructions in the stream beds increase the severity of the flood. Floods also cause alkalinization of the soil (Kerim and Süme, 2019). All rivers have a flood risk area (Figure 1).

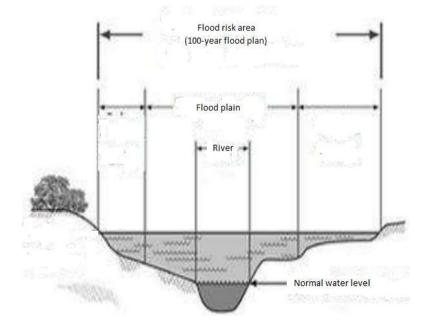


Figure 1. Flood risk area (Kerim and Süme, 2019).

Although floods has benefits such as feeding groundwater, creating fertile land by carrying alluvial soil and benefiting fishing, the harmful effects of floods are more. While the floods cause an annual loss of 100 million dollars in Turkey, the flood investment is only 30 million dollars. Floods can be classified according to the type of precipitation, the duration and time of its occurrence, the place and cause of its occurrence (Kerim and Süme, 2019).

Rain ditches can be used to protect from floods. Rain ditches are divided into two as wet and dry. Wet type in the countryside, dry type in the city should be done. These ditches are important because they purify water as well as reduce flooding. While trapezoidal trenches are used to carry the peak flow rate of 10 years, triangular trenches are used to carry the peak flow rate of 2 years. Rain ditches emerged in the 1970s with the concept of permaculture. Permaculture, a new farming approach, is based on sustainable environmental protection. Rain ditches, it is called as sustainable urban drainage systems in the UK and best management practices in the USA. The main purpose of all of them is reducing the negative effects of impermeable surfaces and benefiting from the rainwater with maximum level. Rather than transmitting water early, water quality should be increased by infiltration and feeding of groundwater should be provided. These conditions should be ensured especially in urbanization area. The dense vegetation of the water ditches also increases the water quality by keeping the pollutants in the water (Ünal and Akyüz, 2017).

The main way to protect from floods is to build a flood protection structure. While the flood protection structures are being built, the slope and width of the stream bed, wall height, construction material and cost should be considered. Moreover, it should be ensured that no excavations are placed in the stream beds and no construction is allowed, zoning plans are made according to the recurring flow rates, and dams should be constructed for sediment retention (Kerim and Süme, 2019).

These precautions should be taken into account especially in big cities such as Istanbul. Istanbul streams, it is known that 73% of they preserve theirs natural structure, 27% turns into a form contrary to theirs nature, 80% passes through the countryside and 20% through the city. While 95% of Istanbul's streams flowing into the Black Sea pass through the countryside, 5% through the city, it is known that 68% of the streams flowing into Marmara Sea pass through the countryside and 32% through the city (Dinç, 2019).

#### Water Harvesting

Water harvesting can be defined as the collection of runoff from precipitation to provide water for the use of all living creatures. This method, it can be used with roof and farm systems in micro watershed size, it can be used as valley bed and out of valley systems in the macro watershed size. Water harvesting is a form of water resources management that collects, stores and organizes rainwater for use. In other words, it is the process of collecting precipitation by flow and storing it for useful use (Yetik and Şen, 2020).

In the water harvesting system, precipitation is collected in the watershed, transmitted to the storage area, and transported to the desired area. water harvest, it is divided into two as micro and macro watershed method. There are valley bed and non valley systems in the macro watershed and farm and roof systems in the micro watershed. Micro watershed system is applied in areas smaller than 0.1 ha, and macro watershed system is applied in large areas. the micro watershed system is more suitable if the watershed slope is up to 50%, and the macro watershed system is more suitable if it is greater than 50%. On a micro scale, 85% of the precipitation falling on roofs can be harvested. On the macro scale, the entrained soil and plants must be cleared if water is to be harvested in the pond. In addition, water should be delivered to the target without increasing infiltration and evaporation (Yetik and Şen, 2020).

Resources can be created for fire, vehicle washing, irrigation and toilet use with the rain water provided from huge structures such as stadium, airport and shopping mall roofs. Especially, the water harvesting systems can be easily applied at homes (Figure 2). Nevertheless, various pathogens can be found in these waters, they need chlorination. Moreover, disinfection can be provided by oxidizing the organic molecule in the water with ozone. The USA, Japan, China, Pakistan and Mexico are developed countries in water harvesting. Tax reductions may be applied to encourage water harvesting and may be made compulsory in the future (Üstün et al. 2020).

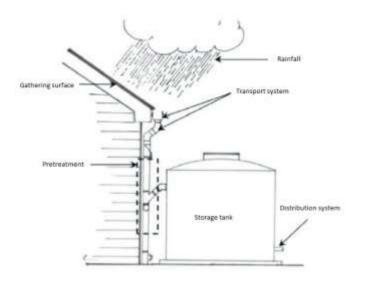


Figure 2. Rainwater harvesting system (Üstün et al. 2020)

#### Water Structures

In order to eliminate the negative effects of surface waters, hydraulic engineering structures should be built in some parts of the road. Hydraulic engineering structures used in forestry applications are cement pipe, culvert, crossdrain and bridge. Among these structures, the most used engineering structures for the removal of water are cement pipe. The cement pipes are structures that generally have circular cross-sections. The main engineering structures used in forest roads are retaining walls, cement pipe (The kind of concrete and iron), crossdrain, culverts, fortification and drainage structures and bridges (Enez and Arıcak, 2010).

Thanks to water structures such as dams and ponds, livestock farming and plant production can be applied easily. If huge water structures are built in transboundary water regions, it may cause conflicts. In particular, water is a strategic issue in the Middle East region. For example, the Euphrates-Tigris Rivers, which are the most important rivers of Turkey, can cause conflicts with neighboring countries in the south because they are transboundary waters. These water resources are also useful Iraq and Syria. The difference between the lowest and highest flows is 28 times for the Euphrates and 80 times for the Tigris. For this reason, especially in dry periods, the importance of these rivers becomes more noticeable (Onuçyıldız et al., 2016). Atatürk Dam on the Euphrates can store water equal to the 3-year flow of the Euphrates with a water storage capacity of 48, Karakaya Dam 9, and Keban Dam 31 billion  $m^3$ . Turkey fulfills its neighbourhood duty against Syria and Iraq and releases at least 500  $m^3$ /s water from the Euphrates River every season. While the water potential in the Euphrates and Tigris watershed constitutes 1/3 of Turkey's river potential, it constitutes almost all of it for Iraq and Syria (Onuçyıldız et al., 2016).

Some of the important water structures on the Euphrates and Tigris Rivers are Ramadi, Fallujah, Kufa, Abbasiye crossbars and Mosul, Duhok, Samarra dams. In particular, the Mosul Dam with a height of 131 m from the foundation is the largest dam in Iraq and the fourth largest in the Middle East region. Samarra Dam has a length of 252 m, a width of 12 m and 17 dam shutter. Duhok Dam has a height of 64 m, a body width of 200 m at the groundwork, 9 m at the crest, and a crest length of 613 m (Onuçyıldız et al., 2016).

#### CONCLUSION

Water is a strategic issue in the Middle East region. Especially, huge water structures in transboundary water regions may cause conflicts. Turkey has huge dams and important transboundary water such as Tigris and Euphrates Rivers. Therefore, water law will remain a critical topic in the region.

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### **CHAPTER 6**

#### NANOTECHNOLOGY AND APPLICATION ON SEEDS

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#### **INTRODUCTION**

The world's population is continually increasing. So that it is estimated that by approximately 2050 the global population will reach around 10 billion. Consequently, global agricultural yields need to increase by 25-30% to be able to feed the growing world population (Scott *et al.*, 2018). To ensure sustainability and enhance food production it is necessary to develop new methods and technologies in agriculture and food industries. To this end, seeds are the genetic resource for plant variety and provide a key factor in any project involving agricultural production and food productivity. Conventional chemical-based agricultural techniques are counter indicated due to the deterioration, hydrolysis, and pollution they create (Umesha *et al.*, 2018). To address these growing issues and repair the ecosystem's damage, it is necessary to create sustainable methods that can support the green revolution (Durán-Lara *et al.*, 2020).

## ROLE OF PRIMING TECHNIQUES IN SEED TECHNOLOGY

The priming of seeds is one of the best ways of promoting rapid and uniform seed germination, seedling establishment and for encouraging plant growth (Ebrahim Pour Mokhtari and Emeklier, 2018,). Seed priming includes hydropriming, halopriming, osmopriming and hormonal priming (Finch-Savage et al., 2004; Ebrahim Pour Mokhtari and Emeklier, 2018; Moameri et al., 2018). Seed priming method is a water-based technique that can promote the germination of aged, damaged, and weak seeds under difficult and adverse ecological conditions (Ebrahim Pour Mokhtari and Emeklier, 2018). Also, it is an innovative sustainable technique, often described as a seed pretreatment method that can increase abiotic stress tolerance in seeds by exposing them to mild stressors or partial hydration with natural or synthetic substances (Ibrahim, 2016; El-Badri et al., 2021). In comparison to unprimed seeds, plants from primed seeds have an increased tolerance against abiotic and biotic stresses, including different germination temperatures, nutrient uptake, water use efficiency, concurrent plant maturity, improves seedling vigor and enhanced plant yield without damaging the ecosystem (Tian et al., 2014; Dutta 2018; Ebrahim Pour Mokhtari and Emeklier, 2018;). This strategy has been shown to be effective when plants are under water stress (Tian et al., 2014; AbbasiKhalaki et al., 2019). In this method, seeds are soaked in a solution with just enough water to promote germination for a period time that is insufficient to allow the radicle to pierce the seed coat (Ebrahim Pour

Mokhtari and Emeklier, 2018; Hu *et al.*, 2020). By employing this technique, seeds are elevated to the germination stage thereby encouraging uniform growth and seed emergence (Bombo *et al.*, 2019).

#### NANOPRIMING

Seed priming techniques may be improved using nanoparticles. Nanoparticles can improve seed germination, seedling growth and crop yield. Nanotechnology employs nanoscale elements and compounds (1 to 100 nm) that affect a wide range of chemical and biological processes. In this regard, nanotechnology has the potential to contribute to the sustainability of agriculture, fill in the gap between atomic structures and bulk materials and revolutionize existing agricultural systems (Fraceto *et al.*, 2016; Maghsoodi *et al.*, 2019).

Of the many engineered nanoparticles that are used in horticulture and agriculture, of particular interest are the nanoparticles of metal oxides that are used in recent versions of some fertilizers, water remediation products, plant protection products, bio-stimulants and herbicides. Also, many nanomaterials have been developed to decrease the supplied amounts of pesticides and plant nutrition products while raising food quantity and quality (Camara *et al.*, 2019; Kah *et al.*, 2019; Zhao *et al.*, 2020). In addition, metal nanoparticles can have positive effects on seeds, plant growth, flowering, agricultural productivity and plant stress tolerance (Acharya *et al.*, 2020; Kah *et al.*, 2019; Lowry *et al.*, 2019; Mahakham *et al.*, 2017; Pérez-de-Luque, 2017, Fraceto *et al.*, 2016).

Nanoparticles can have a range of effects on various plant species. For example, some nanoparticles inhibit root elongation, seed germination or phytotoxicity in seedlings. In contrast to other priming materials, nanoparticles can function as stimulants, enhancing metabolism in seed germination processes, and plant growth by modulating intracellular signal transduction (Falco *et al.*, 2020; Hayes *et al.*, 2020; Pelegrino *et al.*, 2020, Acharya *et al.*, 2020; Mahakham *et al.*, 2017). Leaves can absorb and transport positively or negatively charged nanoparticles to the roots. However, the roots can only absorb negatively charged nanoparticles. Positive charges stimulate the synthesis of mucilage which prevents plant roots from absorbing these nanoparticles (Avellan *et al.*, 2019).

Khodakovskaya *et al.* (2012) first described that how carbon nanotubes (CNTs) can influence germination and growth of tomato seeds. More recently, the use of CNTs has been reported with dicot and monocot

seeds (Mahakham *et al.*, 2017; Anand *et al.*, 2019). Nanopriming is a combination of seed priming and nanoparticle treatment. Particularly in comparison with unprimed seeds or other priming agents, nanopriming is an effective technique for improving seed quality before planting and has the potential to increase crop yields as well as increasing plant tolerance against to environmental stresses (Mahakham *et al.*, 2017; Anand *et al.*, 2019).

There are some major differences between seed priming and seed non-priming. Classic priming methods involves the usage of water; also called hydropriming. Sometimes liquid substances containing nutrients, hormones or biopolymers are used along with water. These may form a coat on the seeds which becomes a protective layer. In the procedure of nano-priming, suspensions or nan formulations are used which are made up of nanoparticles. The seeds are often unable to accept all the nanoparticles (Acharya et al., 2019). In fact, when nanoparticle absorption occurs, the majority of absorption is kept as a coating on the seed exterior (Mahakham et al., 2017; Duran et al., 2017; Falsini et al., 2019; Montanha et al., 2020). This coating can work as a protective layer from external bacteria, fungi and other pathogens. In order to succeed in the experiment, it depends on the size, concentration, zeta potential and other physical-chemical properties of the nanoparticles. The surface charges of nanoparticles have a major effect in determining their influence, biological responses and susceptibility of plants species (Acharya et al., 2019; Pérez-de-Luque, 2017, Bombo et al., 2019; Hu et al., 2020; Palocci et al., 2017; Valletta et al., 2014).

Recently, seed pretreatment agents have been shown to promote seed germination, seedling growth and plant stress tolerance. Several metal-based NPs (e.g., AgNPs, AuNPs, CuNPs, FeNPs, FeS<sub>2</sub>NPs, TiO<sub>2</sub>NPs, ZnNPs, ZnONPs) and carbon-based NPs (e.g., carbon nanotubes) promote seedling growth and plant stress tolerance and thus subsequently enhance the plant yield and the nutritional value of the food from some crop plants (Acharya *et al.*, 2019; Wang *et al.*, 2020; Ye *et al.*, 2020; Pereira *et al.*, 2021; Shah *et al.*, 2021).

With the seed nano-priming technique, only a few researchers have suggested that the primed seeds must reach their original moisture content before planting (Ebrahim Pour Mokhtari and Emeklier, 2018). Soaking seeds in nanoparticles and drying them causes a priming memory in seeds which can be recruited after further stress exposure and promotes better stress tolerance during nano-primed seed germination. Since the nano-priming technique is relatively new (Acharya *et al.*, 2019), the physiological and molecular mechanism(s) behind this technique and the ability of NPs to promote seed germination, modify seed metabolism and plant protection products require additional research.

#### **MECHANISM OF NANOPRIMING TECHNIQUE**

Nano-priming techniques cause some physiological and biochemical changes in seeds. Nano-primig stimulates up-regulation aquaporin genes in response to an external stimulus and leads to the production of nanopores on seed coats which assist seed nutrient and water absorption. Absorption of water activates ROS (reactive oxygen species) systems which serve as signaling molecules to start metabolic processes related to germination. The reduction of  $O_2^{-}$  (superoxide radical) to  $H_2O_2$  (hydrogen peroxide), diffusion of  $H_2O_2$  to the embryo and finally the interplay between  $H_2O_2$  and gibberellic acid (GA<sub>3</sub>) is catalyzed by superoxide dismutase (SOD). GA<sub>3</sub> activates  $\alpha$ -amylase synthesis for hydrolysis of starch which is converted into soluble sugars, thinning of cell walls, and acceleration of the growing of embryo resulting in more rapid root development (Tripathi *et al.*, 2017; Abdel Latef *et al.*, 2018; Shang *et al.*, 2019; Pereira *et al.*, 2021; Khalaki *et al.*, 2021; Rai-Kalal *et al.*, 2021).

#### FACTORS AFFECTING NANO-PRIMING

It is necessary to consider different factors that influence the effectiveness of nano-priming. The kind and the size of the NPs that affect plant germination depend on several interrelated parameters, such as plant species, soil water content, temperature, the type and NP concentration, the length of contact, the properties of the nanomaterials and the interaction between plants and their living environment (Ma *et al.*, 2010; Miralles *et al.*, 2012).

#### EFFECT OF TEMPERATURE ON NANO-PRIMING

The main purpose in seed priming techniques is to reduce the period that the radicle needs to penetrate the seed coat. This period depends on the priming agents and type of plant. Generally, room temperature is suitable for most plant species (Anand *et al.*, 2020; Sarkar *et al.*, 2020; Guha *et al.*, 2021). Lower, or higher temperatures are generally undesirable for older seeds because high temperatures can deactivate the enzymes that are necessary for the germination process.

## EFFECT OF KIND AND CONCENTRATION OF NANOPARTICLES ON NANO-PRIMING

The rate of nano material absorption into the seed is influenced by the kind and concentration of nanoparticles. When rice seeds were primed with different nanoparticles, the optimum dosage of ZnO NPs was 10 mg L<sup>-1</sup>, while the optimum concentration of Fe NPs is 40 or 80 mg L<sup>-1</sup> (Itroutwar *et al.*, 2020; Guha *et al.*, 2021). Also, based on the rate of germination of various seeds, the highest germination rate was obtained from a medium amount of these compounds. Additionally, it is important to remember that plants produced through nano-priming must be harmless. Thus, absorption of high levels of nanoparticles may be toxic to the plant (Song and He, 2021).

#### LENGTH OF NANO-PRIMING

Length of nano-priming is dependent on plant species, type of nanomaterial, priming methods and temperature. During the priming period it is important to ensure that the seeds are thoroughly soaked without germination. In the dry treatment method, seeds are exposed to nanoparticles for approximately 15 hours, whereas in the wet treatment method seeds are soaked in a nano solution for three hours (Hossen *et al.*, 2020).

## LIMITATIONS OF NANO-PRIMING TECHNIQUES AND FUTURE PROSPECTS

Despite significant progress with this technology, a complete understanding of how nanoparticles effect the biology of seeds are still not very clear. There are only theoretical projected levels of exposures to the nanoparticles. The fact that there is not a clear understanding of how nanoparticles affect the environment, and the health and safety of both human and non-human participants is still a matter of huge concern (Kashyap *et al.*, 2015). There isn't a standard principle for seed nano-priming. Additionally, there is no obvious pattern in the priming responses according to the species' taxonomic position. Moreover, some nano-priming treatments have the potential to be contaminated with bacteria and/or fungi, which might seriously impair seed germination (Panpatte *et al.*, 2016).

After nano-priming, seeds must be dried to their initial moisture content. This procedure is usually faster compared to dehydration of mature seeds (Panpatte *et al.*, 2016). According to several researchers, intense desiccation techniques change the effects of nano-priming (Marthandan *et al.*, 2020). This process may result in less stability of the nano primed seeds and

lead to additional costs for companies and farmers. In some circumstances, repeating nano-priming can help seeds to avoid losing viability, while in other cases, losses are irreversible and permanent (Chandra *et al.*, 2015). While additional treatment is possible, besides creating an additional cost, it may not completely restore the germination capacity of the treated seeds.

#### CONCLUSION

Overall, using nanoparticles in seed technology is in an early stage of development. Treatment seeds with nanoparticles is one strategy that has the potential to move conventional agriculture to sustainable agriculture. However, the present research on this subject is insufficient. Thus, there are still a lot of unknowns regarding the ecotoxicity, acceptable limit, and absorption capability of various nanomaterials. It is therefore necessary to continue research considering the types of nanoparticles, the domain of nanoparticle, the duration of their action, the effect of plant species and soil conditions. Moreover, this research should investigate which organs of the plant are primarily affected by nanoparticles to promote or inhibit growth and development.

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# **CHAPTER 7**

# EFFECTS OF FILM COATING ON SOME PHYSICAL PROPERTIES OF OKRA (Abelmoschus esculentus) SEEDS

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#### **1.INTRODUCTION**

Okra is one of the oldest cultivated plants which is from the hibiscus family. Okra (*Abelmoschus esculentus*), known to be of Ethiopian origin, has spread over a wide geography from Africa to Asia, from Southern Europe to America since the beginning of the 12th century.

Okra is grown commercially in subtropical, temperate and Mediterranean climate zones. Such as India, Japan, Turkey, Iran, West Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, India, Brazil, Ethiopia, Cyprus, USA (Qhureshi, 2007; Gemede, et al., 2014) (Figure 1).

In Turkey, 8.799 tons of products were obtained from an area of 6.047.980 da in 2005; 8.247.642 tons of products from 5.623.673 da in 2010; 23.136.971 tons from 5.052.874 da in 2015 and 23.147.529 tons of from 6.060.162 da product obtained in 2018 (Yanmaz et al., 2020). The increase in both the production area and the amount of production in Turkey over the years shows that the interest in okra and the possibilities for its use have also increased.

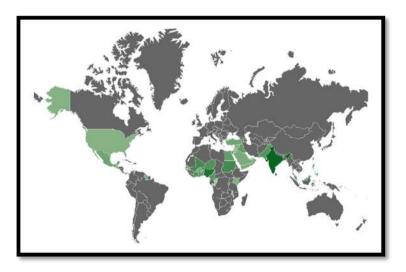


Figure 1. Places where okra grows in the world

Almost all of the okra plant (fresh leaves, buds, flowers, pods, stems and seeds) are evaluated in different ways (Mihretu et al., 2014) (Figure 2). Okra is a plant used not only as an agricultural product but also in medical and industrial applications (Madison, 2008).

Okra is rich in calcium, potassium and other minerals and vitamins. In addition, due to the amount of protein and fat it contains (approximately 20-40%) and its quality, it has started to be preferred more and because of the contributions it will make to human nutrition. Especially the high rate of unsaturated fat comes to the fore with its important and positive effect on cardiovascular diseases (Sorapong, 2012; Kumar et al., 2013). For this reason, the food industry offers okra to consumers in different forms (fresh, cooked, in soups or salads, canned) (Doymaz, 2005). Composition of okra pods (81% of product purchased, ends trimmed) per 100 g edible portion of okra: water 88.6 g, energy 144.00 kJ (36 kcal), protein 2.10 g, carbohydrates 8.20 g, fat 0.20 g, fiber 1.70 g, Ca 84.00 mg, P 90.00 mg, Fe 1.20 mg,  $\beta$ -carotene 185.00 µg, riboflavin 0.08 mg, thiamine 0.04 mg, niacin 0.60 mg, ascorbic acid 47.00 mg (Benchasri, 2012).



Figure 2. Okra (Abelmoschus esculentus) (Kumar et al., 2013)

Okra is a culture plant suitable for cultivation in large areas. For this reason, researches are carried out for better, quality and more production. As it has a sensitive structure, it has been determined as a result of studies that its tolerance to salty soils, drought conditions and low temperature is not good (Kuşvuran, 2011; Kumar et al., 2013). However, on the other hand, it has

been stated that it can grow up to 2.5 m depending on the climate and soil characteristics and its yield values change (Elmacı, 2011). Especially since okra is affected by soil temperature, Robbins (1982) stated that okra seeds can be planted around 18°C; Fasheun (1988) pointed out that okra needs higher soil temperature (35°C) than other cultivated plants. Of course, the effect of the okra variety to be grown here should also be considered (Düzyaman et al., 2004). Local populations with many different characteristics are grown in Turkey (Vural et al., 2000; Düzyaman & Vural, 2002).

Each seed has its own characteristic features. Although these can change according to climate and environmental conditions, they are included in the basic information about seeds. These data are always evaluated in the development of new varieties, especially in breeding studies and in increasing their resistance to ecological conditions. In addition to these, mechanization practices are used in order to grow the products at the targeted level on large lands. These parameters are also evaluated in the selection of the appropriate planting machine and the planting arrangement. In particular, it helps the producers to produce in more economical conditions by preventing adverse situations (twining-gap) that may occur during planting.

Seed technology is used for a healthy and high quality production by improving seed characteristics. Film coating application is one of them. Hormones, drugs, etc. that the seeds need. products such as can be applied to the seeds before planting with this method. In this way, seeds can be protected against many conditions such as improving the germination abilities of seeds, ensuring storage resistance, and preventing negative situations that can be experienced with insects and pests.

In this study, some physical properties of the seeds of okra plant, whose production amount has increased in recent years and evaluated by different sectors, were determined. It is aimed that the obtained values will contribute positively to the improvement studies and mechanization and production.

#### 2. MATERIAL AND METHOD

This study was carried out at Bingol University Faculty of Agriculture Biosystem Engineering laboratories in 2022. The okra seeds (belonging to the 2021 harvest) used in the study were obtained from a commercially available company. In the study, water based polymeric material (commercially available) was used as the film coating material and was applied on the seeds by spraying as a single layer. Untreated okra seeds accepted as the control group and film-coated okra seeds, 100 seeds were selected randomly with using a stereo microscope (Nikon SMZ 745T) with its own software and the length (mm), width (mm), surface area of the seeds and area data (mm2) measured (Dumanoğlu & Öztürk, 2021; Dumanoğlu et al., 2022). Using the values obtained from here, the average arithmetic diameter of the seeds (L+W)/2) (mm), the average geometric diameter ((L\*D^2)<sup>1/3</sup>) (mm), sphericity (D<sub>0</sub>/L) data formulas help (L: Seed length value (mm) W: Seed width value (mm), D: Average arithmetic diameter (mm); D<sub>0</sub>: Average geometric diameter (mm)) (Mohsenin, 1970; Alayunt, 2000; Kara, 2012. After all these procedures, the seeds were weighed randomly and in four repetitions on a 0.001 g precision balance (Dumanoğlu & Geren, 2020). The obtained value was evaluated according to the basic statistical parameters.

#### **3. RESULTS**

In the study, some physical properties of the control group and filmcoated okra seeds were determined. When we examine the seeds together; In general, it was determined that they had an average length of 5.229 mm, a width of 4.476 mm, a surface area of 20.282 mm<sup>2</sup>, an arithmetic diameter of 4.852 mm, a geometric diameter of 41.255 mm and a sphericity of 7.861. Thousand grain weights were weighed as 5.580 g. On the other hand, it was determined that the film-coated okra seeds had higher values than the control group, although not very significant (Figure 3). The data obtained are consistent with the values in the study of Sahoo & Srivastava (2002) (5.92 to 7.30 mm length; 4.71 to 5.40 mm width).

Okra plant is an important commercial culture plant that is used not only in human nutrition, but also by many sectors, from medicine to biodiesel material, and almost completely. Since it has a suitable structure to be grown in large areas, researches are carried out to improve the breeding and growing conditions of this plant. In this study, it was carried out especially for the sowing of okra seeds on larger lands with the help of mechanization. In addition to these, it is thought that the specified seed characteristics will help researchers who are doing breeding studies.

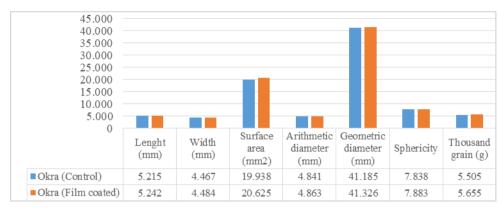


Figure 3. Some physical properties of okra seeds (Control and film coated)

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# CHAPTER 8

#### DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF SOME WALNUT VARIETIES CULTIVATED IN TUNCELI PROVINCE

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#### **INTRODUCTION**

Walnut is a fruit belonging to the *Juglans genus* of the *Juglandaceae* family. Walnut fruit, which is widely cultivated in different regions of the World, has an important role on human health due to its rich nutritional content (Carvalho et. al., 2010). Walnut is referred in the group of hard-shelled fruits and its fruit consists of green shell, hard Shell and kernel on the tree (Arabacioglu, 2017). According to FAO, walnut cultivation is in the top three with a share of 9.3% in the World and in the first place with 25.8% in production per year. The People's Republic of China is the largest walnut producing country in the world, with an annual production of approximately 1.5 million tons. The People's Republic of China is followed by the United States and Iran in terms of annual walnut production, respectively. Republic of Turkiye meets approximately 10% of the total walnut production in the World every year, in this case, it makes Republic of Turkiye the 4th largest walnut producer country (TUIK, Date of Access: 1.8.2022).

Walnut trade in the World is taken place with or without Shell. Walnut Shell constitutes approximately 40- 55% of walnut product. Until recently, walnut shells were considered as food waste. However, in various previous studies, products with commercial value have been generated using walnut shells (Arabacioglu, 2017; Arinci et. al., 1998; Akgol et. al., 2015; Yildirim et. al., 2022). The part that has the main commercial value in the walnut fruit is its kernel in inner part of the shell. Walnut kernel is a recommended food source due to its rich bioactive elements. The reason for this is that walnut kernel contains protein close to the protein content found in meat and meat products (Ayaz, 2008) and approximately 70% of the total fat content of walnut kernel is polyunsaturated fatty acids, approximately 20% is monounsaturated fatty acids and approximately 10% is saturated fatty acids (Baysal, 1991). In addition, walnut kernels contain high amount of vitamin E and other antioxidants (phytosterols and polyphenols). In clinical studies, it has been reported that regular walnut consumption significantly reduces the risk of cardiovascular diseases and cancer in individuals (Anderson, 2011).

Republic of Turkiye has a great potential for walnut cultivation due to its ecological richness. It has even been stated that walnut cultivation in Republic of Turkiye dates to 3000 years ago (Akca, 2009). Walnut farming is widespread in Republic of Turkiye, even some regions in Republic of Turkiye (such as Ermenek, Kahramanmaras, Bitlis, Erzincan, Niksar, Kaman) are known for the walnuts grown (Kapluhan, 2015). Even though climate characteristic makes walnut farming possible and common in Tunceli, recognition of walnut varieties harvested in Tunceli every year is very low. The walnut farming in Tunceli gets more attention day by day due to high altitude of walnut cultivation in Tunceli (Anonim, 2016). In this research, pomological characteristics of walnut fruits and kernels harvested in Ovacik, Nazimiye and Hozat districts of Tunceli in 2021 were determined for the first time. Moreover, the physicochemical properties of walnut kernel varieties were investigated. To this respect; the moisture content, ash rate, acidity, oil rate and protein content of walnut kernel varieties were determined and statistically evaluated.

#### SAMPLE PREPARATION PROCESS

Samples were obtained from walnut producers in Ovacik, Nazimiye and Hozat. In order for the samples to fully represent the walnut mass, walnut fruit was harvested from at least 4 trees in the producer's garden. After the samples were taken from the manufacturer, all of them were brought to Munzur University Food Engineering laboratory and they kept in dark at  $20^{\circ}$ C until the analysis start off.

Walnut fruits taken from the producer were mixed and 10 of them were randomly selected to determine the pomological characteristics of Tunceli walnut kernel varieties. For physicochemical analyses, the outer shell of the walnut was broken, and the kernel part was taken away. For each walnut variety, at least 250 g of each walnut kernel was weighed then the weighed kernels were pulverized and mixed. Mixed walnut kernel powder was used in the analyses.

### METHOD

### **Determination of Pomological Characteristics**

Pomological characterization was performed on 10 walnut fruits randomly taken from different trees. The weight of the fruit with its shell (g), the weight of kernel that comes out of the fruit (g) and the yield ratio were investigated (Yarilgac, 1997).

#### **Moisture Content**

10 g sample was dried in an oven at  $105^{\circ}$ C until it reached constant weight, then cooled in a desiccator (Cemeroglu, 2010).

### Ash Content

Ash content was determined using the AOAC<sup>1</sup> metod. 3 g of sample was weighed into the crucibles, after waiting for 1 hour in the oven, the burning process was carried out at  $600^{\circ}$ C in the muffle furnace until the inside of the crucible turned white. The crucibles were cooled in desiccator, then weighed.

## Acidity (pH)

The method used in the study of Kalkan et. al. (2012) adapted for this study. 5 g sample was mixed with 50 mL distilled water for 2 hours using a magnetic stirrer, then the mixture was filtered, and 20 mL was taken from the filtrate. Acidity was determined in a calibrated digital pH meter.

### **Oil Content**

The oil content in Tunceli walnut kernel varieties were determined by using the Soxhlet method which is explained in the study of Dogan and Basaoglu (1985). 10 g of powdered walnut kernel was weighed into Soxhlet cartridge. N-hexane was used for the extraction process. The extraction process was decided to be 5 hours (Aydin, 2022).

### **Protein Content**

Protein content was determined by Kjeldahl method (AOAC<sup>2</sup>). 1 g of powdered walnut kernel was weighed, and 25 mL sulfuric acid and catalyst tablet were added to it. The burning process was continued until green colour was formed. After the tubes cooled, the distillation process was started. Titration was done using 0.1 N HCI, crude protein content of walnut kernel varieties was determined according to the following formula;

% N = 
$$\frac{[(V_1 - V_0)x N x F x 14 x 10^{-3}]x 100}{M}$$

 $V_1$  = Volume of spent HCI acid solution (mL)

 $V_0$  = Volume of HCI acid solution spent in the witness experiment (mL)

N = Concentration of adjusted hydrochloric acid solution (0.1)

M = Weight of sample taken (gr)

F = Nitrogen to protein conversion factor (6.25)

### **Statistical Analysis**

All the analyses of walnut kernel varieties were done in three parallels according to a randomized block design. SPSS 22 program was used to evaluate results of each analysis. Kolmogorov- Smirnov and Shapiro- Wilk tests were utilized to determine whether results for each analysis show homogeneous distribution. It was found that each analysis data set showed a homogeneous distribution, hence parametric statistical methods were decided to be used for evaluation. In variance analysis, one-way ANOVA and Duncan multiple comparison test (0.05%) were used for significance levels. Pearson test was used for correlation statistics.

### **RESULTS AND CONCLUSION**

	Fruit Weight	Kernel Weight	Yield
	(g)	(g)	(%)
Ovacik	$9.39 \pm 1.51 b$	$4.23\pm0.98b$	$44.73\pm3.29$
Hozat	$11.83 \pm 1.04b$	$5.08\pm0.45b$	$42.93\pm0.55$
Nazimiye	$19.80 \pm 1.39a$	$9.67\pm0.61a$	$48.94\pm3.99$
Significance	< 0.001	< 0.001	0.115

Table 4.1. Pomological characteristics of Tunceli walnut kernel varieties.

Evaluation of pomological characteristics of Tunceli walnut fruit and kernel varieties harvested in 2021 is demonstrated at Table 4.1. It was found that fruit weight was 9.39-19.8g, kernel weight was 4.23-9.67g and yield was

between 42.93- 48.94%. In previous studies investigating the pomological properties of walnut fruit and kernel, it was stated that fruit weight ranges from 6.89 to 18.79 g (Akca and Koroglu, 2005; Bakkalbasi et. al., 2010; Keles, 2012; Koyuncu and Gorgun, 2003; Ozkan, 2002; Paunovic, 1990; Revin, 1990; Yarilgac et. al., 2002), kernel weight was 3.02- 8.58 g (Akca and Koroglu, 2005; Bakkalbasi et. al., 2010; Keles, 2012; Koyuncu and Gorgun, 2003; Ozkan, 2002; Yarilgac et. al., 2002), yield was in between 41.25- 64,5% (Bakkalbasi et. al., 2010; Keles, 2012; Ozkan, 2002; Paunovic, 1990; Revin, 1990; Yarilgac et. al., 2002). It was found that even though the yield of Tunceli walnut kernel varieties doesn't show any significant difference, fruit weight and kernel weight changed significantly dependent on variety of walnut kernel in one-way ANOVA test. To determine which variety has difference among samples, Duncan test conducted, the result of the test showed that the fruit and the kernel of Nazimiye walnut is significantly higher than the weight of the fruits and kernels of other walnut varieties, and no significant difference between the weight of fruit and kernel of Hozat and the weight of fruit and kernel of Ovacik was found. Although there is a significant difference in fruit and kernel weights of Tunceli walnut cultivars, it is noteworthy that no significant difference was found in their yield.

Possible correlations among the weight of fruit, kernel and yield were analysed. It was found that there was a strong correlation between walnut fruit weight and yield (r= 0.614), when there was a very strong correlation between walnut kernel weight and yield (r= 0.744). This result is in agreement with the result of Leahu et. al. (2013). Leahu et. al. (2013) stated a strong positive correlation between kernel weight and yield for 9 walnut kernel varieties harvested in Suceava, Romania. This can be interpreted that the yield in walnut farming is not related to the altitude of the grown region but is related to the weight of the grown kernel variety (Tunceli altitude: 922 m, Suceava altitude 271 m).

	Moisture	Ash Content	Acidity (pH)	Oil Content	Protein Content
	Content				
Ovacik	$3.53 \pm$	$1.17\pm0.28$	$5.88\pm0.13$	$77.85\pm2.16a$	$16.21\pm0.33a$
	0.28a				
Hozat	$2.56 \pm$	$1.39\pm0.14$	$5.90\pm0.07$	$66.81\pm0,75b$	$14.70\pm0.64b$
	0.11b				

Yable 4.2. Physicochemical properties of Tunceli walnut kernel varieties.
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Nazimiye	$2.95 \pm$	$1.31\pm0.14$	$5.71\pm0.07$	$67.18 \pm 1.45 b$	$13.13\pm0.07c$
	0.26b				
Significance	0.006	0.45	0.102	< 0.001	< 0.001

Moisture ratios of Tunceli walnut kernel varieties were determined in the range of 2.56- 3.53%. Previous studies reported that moisture content of walnut kernel varieties was in the range of 2.29- 4.92% (Al- Bachir, 2004; Bakkalbasi et. al., 2010; Caglarirmak, 2003; Erkol et. al., 2008; Payne, 1985; Ruggeri et. al., 1998). Therefore, the result of this study is compatible with previous studies in the literature. However, Savage (2001) found 5.1- 6.7% moisture content in walnut kernel and Ozrenk et. al. (2005) stated the moisture content of apricot kernel harvested in Erzincan is in range of 2.19-5.33%. Oneway ANOVA test is done to find out whether there is a significant difference in moisture content among walnut kernel varieties and the result showed that the moisture content changed significantly in regard to walnut kernel variety. To determine which variety has difference among samples, Duncan test conducted, the result of the test showed that Ovacik variety has higher moisture content than other walnut kernel varieties when no significant difference found in moisture content between Hozat and Nazimiye walnut kernels. It could explain why the results of Savage (2001) and Ozrenk et al. (2005) were different. In addition, Erkol et. al. (2008) and Bakkalbasi et. al. (2010) found similar result with this research that moisture content could statisticially change among walnut kernel varieties. Erkol et. al. (2008) determined that the moisture content of Bilecik is 4.22% when Yalova walnut kernel has 4.92% moisture content. Bakkalbasi et .al. (2010) stated the moisture content of 7 different walnut kernel varieties (Yalova 1, Yalova 3, Yalova 4, Kaman V, Sebin, Bilecik and Sen 1) harvested in 2004 is in range of 2.29- 3.33%. It is interesting that different studies as to walnut kernel's moisture content found different moisture content for the same walnut kernel type. It might be raised due to the different way of caring for walnut fruit or climate difference during growing of walnut fruit (Katar et. al., 2012; Yilmaz and Tuncturk, 2018).

Ash ratios of Tunceli walnut kernel varieties were found in the range of 1.17-1.39%. In previous studies, ash content in walnut kernel varieties was reported to be in the range of 0.77-2.8% (Akca, 2000; Akca and Koroglu, 2005; Bakkalbasi et. al., 2010; Beyhan, 2005; Koyuncu et. al., 2003; Ozkan, 2002; Ozrenk et. al., 2005; Sen, 1980; Yarilgac et. al., 2002). Therefore, it could be stated that the result of this study is compatible with the previous studies in the literature. Ash content in Tunceli walnut kernel varieties didn't statistically

differ according to one-way ANOVA test. This finding is parallel to the result of Koyuncu et. al. (2003) who studied ash ratios of 20 walnut kernel varieties harvested in Van/ Bahcesaray. In addition, it is noteworthy that different ash ratios were explained in the same walnut kernel variety in the studies (Akca and Koroglu, 2005; Sen, 1980; Yarilgac et. al., 2002). It could be due to season in which walnut kernel varieties were harvested (Akca, 2000). Akca (2000) used four different walnut kernel varieties harvested in Niksar district of Tokat in 1997- 1998. He expressed that ash content in walnut kernel varieties differ in according with fruit development period and ash content in Sebin walnut type was in range of 1.7-2.2%, ash content in Bilecik walnut type was in range of 2.07- 2.8% and ash content in 32 B-18 walnut type was in range of 2.15- 2.69.

The acidity of Tunceli walnut kernel varieties was found in pH range of 5.71- 5.9. Bakkalbasi et. al. (2010) studied acidity of 6 walnut kernel varieties (Yalova 1, Yalova 3, Yalova 4, Sebin, Bilecik, Sen 1, Kaman 5). Bakkalbasi et. al. (2010) reported that acidity of these walnut kernel varieties didn't change significantly and was in the range of 5.77- 6 pH. The finding of Bakkalbasi et. al. (2010) is in agreement with this study's result.

Previous studies which researched oil content in walnut kernel varieties stated that walnut kernel varieties have oil content in range of 48-97-75.61% (Akca and Koroglu, 2005; Batun, 2005; Beyhan, 2005; Gao et. al., 1991; Kahraman, 2006; Koyuncu et. al., 2003; Ozkan, 2002; Ozrenk et. al., 2005; Revin,1990; Simsek, 2010; Yarilgac et. al., 2002; Yerlikaya et. al., 2012). In this study, oil ratios of Tunceli walnut kernel varieties were found to be 66.81-77.85% and even though oil ratios of Hozat and Nazimiye walnut kernel type are in accordance with the literature, Ovacik walnut kernel draws attention in terms of the highest oil content found in walnut kernel varieties until now. This difference can be interpreted as genotype difference of Ovacik walnut kernel variety (Akbari et. al., 2012; Korekar et. al.; 2011). One-way ANOVA test is done to find out whether there is a significant difference in oil content among walnut kernel varieties and the result showed that the oil content changed significantly in regard to walnut kernel variety. This result is parallel to the result of Koyuncu et. al. (2003) who analysed oil content of 20 walnut kernel varieties harvested in Van/ Bahcesaray. However, some of these 20 walnut kernel varieties in the study of Koyuncu et. al. (2003) didn't statistically differ from each other. This may be caused by many factors. These factors could be genotype similarity (Gore and Kurt, 2021; Sathe et. al., 2008), similarity of soil structure in which walnut varieties are grown, similar maintenance conditions or even similar climate conditions (Ekren et. al., 2007; Katar et. al., 2012; Yilmaz and Tuncturk, 2018). To determine which variety has different oil content among Tunceli walnut kernel varieties, Duncan test conducted, the result of the test showed that Ovacik walnut kernel had higher oil ratio than that in Hozat and Nazimiye walnut kernel varieties.

Protein ratio in walnut kernel varieties was stated to be found between 11.14- 23.43% by previous studies (Akca and Koroglu, 2005; Arda, 2006; Beyhan, 2005; Guven, 2000; Kazankaya et. al., 2003; Keles, 2012; Koyuncu et. al., 2003; Leahu et. al., 2013; Oguz and Askin, 2007; Ozkan, 2002; Ozongun, 2001; Ozrenk et. al., 2005; Unver and Celik, 2005; Yarilgac et. al., 2002, Yildirim et. al., 2005). Contrary to these, Kahraman (2006) found up to 27.5% protein content in walnut kernel varieties harvested in Agacoren district of Aksaray, Beyhan (2005) stated 11.14% protein content in walnut variety harvested in Darende district of Malatya, Akca and Koroglu (2005) expressed 9.87% protein content in walnut kernel type (19.12.12) which was grown in Iskilip district of Corum. In this study, protein content ranging from 13.05% to 16.58% in Tunceli walnut kernel varieties is consistent with most of the studied who expressed walnut kernel varieties' protein content in the literature. Oneway ANOVA test is done to find out whether there is a significant difference in protein content among walnut kernel varieties and the result showed that the protein content changed significantly in regard to walnut kernel variety. To determine which variety has different protein content among Tunceli walnut kernel varieties, Duncan test conducted, the result of the test showed that Ovacik walnut kernel type has the highest protein content when the lowest protein content was found to be in Nazimiye walnut kernel type. This finding could explain remarkable differences of the results of Kahraman (2006), Beyhan (2005), Akca and Koroglu (2005) studies compared to the results of other walnut kernel results in literature. Additionally, although Agacoren district which has the highest altitude (1100 m) among studies in literature was found to have the highest protein content, it is noteworthy that Nazimiye district which has the highest altitude (1550 m) among Tunceli walnut kernel varieties has the lowest protein content in walnut kernel varieties studied in this research. This is proof that soil structure, climate conditions and walnut fruit's care throughout cultivation can affect on protein content of walnut kernels (Ekren et. al., 2007; Katar et. al., 2012; Yilmaz and Tuncturk, 2018). Furthermore, the genotype feature in fruits' kernel influences protein content (Gulsoy and Balta, 2014). Overall, it can be concluded that the high altitude in the region where

the walnut fruit are grown doesn't affect on protein content of walnut kernel varieties.

All in all, Tunceli walnut kernel varieties harvested in Ovacik, Nazimiye and Hozat in 2021 were analysed in this research, pomological characteristics of these walnut varieties were found as fruit weight 9.39-19.8g, kernel weight 4.23-9.67g and yield 42.93-48.94%. Although weight of walnut fruit and kernel harvested in Nazimiye are heavier than weight of fruit and kernel of other walnut varieties, it is worth to note that there is no significant difference in yield ratio among Tunceli walnut kernel varieties.

The chemical properties of Tunceli walnut kernels were found as 2.56-3.53 % in moisture content, 1.17- 1.39 % in ash content, 5.71- 5.9 pH in acidity, 66.81- 77.85 % in oil content and 13.13- 16.21 % in protein content. Although the ash ratios and acidity don't significantly differ among the analysed walnut varieties, finding of significant difference in moisture content, oil content and protein content draw attention. The finding means that it is possible to be difference in composition of phenolic content and peptide sequences among Tunceli walnut kernel varieties. Therefore, the effect of walnut variety on oil composition, total phenolic content, antioxidant content, antimicrobial activity and bioactive peptide sequences need to be examined.

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# **CHAPTER 9**

#### QUALITY ATTRIBUTES OF SOME WINE GRAPE CULTIVARS GROWN IN MALATYA PROVINCE AND RELATIONSHIPS BETWEEN THESE ATTRIBUTES

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#### **INTRODUCTION**

Wine is produced in several countries of the world. European Union countries play a significant role in world wine market (Tosun, 2005). The greatest wine producer countries of the world are ordered as Italy (48.8 mhl), France (41.9 mhl) and Spain (37.8 mhl). Besides these countries, the USA (22.5 mhl) and Australia (12.5 mhl) are also among the leading wine producers of the world. World annual wine production was around 259.6 million hectoliters in 2016 (Anonymous, 2017). Turkey with 468 thousand hectares of vineyard sites has the 5th place in world vineyards and with 4 million tons of annual grape production has the 6th place in world grape production. Considering the income earned from grapes, Turkey has a quite low share in wine production (about one per thousand) which is the most value product produced from the grapes. Only 1% of annual production is processed into wine in Turkey. France with almost equal grape production levels with Turkey earns about 10 billion \$ form wine export. The income of Turkey from wine production is about one per thousand of France. Turkey has a great potential in this sector (Anonymous, 2016). Such a potential should be activated through well analyses of the current cases. Wine production is a profitable commercial activity with a great valueadded and Turkey has a significant potential to be a leading country in wine production. Therefore, this production sector should be supported (Tosun, 2005). The net profit in wine grape facilities was reported to be 59.5% greater than the table grape producer facilities (Bayramoğlu et al., 2010). Ecological conditions of Turkey offer perfects options for quality wine production (Celik et al., 2005; Tangolar et al., 2005). Especially entire Tracia region, some sections of Aegean region, dry sections of Central Northern, Central Eastern and Southeastern sections with hot and dry summers and cool nights allow sufficient sugar accumulation, high acidity, aromatic substance and tannin contents in berries all sufficient for quality wine production (Celik et al., 2005; Tangolar et al., 2005). There are various studies carried out about quality attributes of local and foreign wine grape cultivars in Turkey (Köylü et al., 2002; Tangolar et al., 2002; 2005; Deryaoğlu and Canbaş, 2003; Soyer et al., 2003; Cangi et al., 2009; 2011; Gök Tangolar, 2009, Gök Tangolar et al., 2009; Özden and Vardin, 2009; Kamiloğlu and Üstün, 2014). The table wines obtained from hot regions usually have low quality. Phenolics and especially the aromatic compounds of the must obtained from the grapes of hot regions are significantly lower than the ones obtained from the grapes of cold regions. Although grapes are ripened faster in hot regions, anthocyanins accumulation

decrease significantly and in most cases fruit color is not sufficient to produce quality red wines (Lavee, 2000).

Öküzgözü is the most valuable red wine grape cultivar of Anatolia. It has a intense aroma and are remarkable with its light ruby color. It is commonly produced in vineyards of Elazığ and Malatya provinces of Turkey. On the other hand, Boğazkere cultivar grown in Diyarbakır province is quite rich in tannin (tannic acid) and offers a soury taste to wine lovers. The wines produced with the mixture of Öküzgözü and Boğazkere cultivars are the most famous and preferred wines of Turkey. In Eastern and Southeastern Anatolia regions, Midyat and Mardin have been known with their wine cultures for centuries. The wines of these districts are usually produced by Assyrians of which the numbers are continuously decreasing. These wines are mostly produced from local cultivars of Mazrone and Kerküş (Ağaoğlu et al., 1995; Çelik, 2007). Throughout Turkey, the grape cultivars of Narince, Öküzgözü, Boğazkere, Cabernet Sauvignon, Kalecik Karası, Sirah, and Merlot are prominent wine cultivars (Söylemezoğlu et al., 2010).

In this study, quality attributes of Öküzgözü, Kalecik Karası, Hasandede and Kabarcık grape cultivars grown in Malatya province were investigated and the relationships among these attributes were assessed.

### MATERIAL AND METHOD

This study was conducted at producer vineyards of Banazı village of Malatya province with four different grape cultivars (Öküzgözü, Kalecik Karası, Hasandede, Kabarcık) in 2015. Vines were planted at 1.5 x 3 m spacing and cordon trained 50 cm above the ground. While the performance of yield pruning, vines were loaded as to have 20-22 buds/vine. Basic fertilizers were applied to experimental vineyards and vines were irrigated with drip lines. Tipping and topping processes were separately performed.

Experiments were conducted in "randomized plots design" with 3 replications. In replicate, 15 clusters were used. Cluster width (cm), cluster length (cm), cluster weight (g), cluster volume (ml), cluster size (width x length) (cm<sup>2</sup>) of the cultivars were investigated. From about 1/3 section of each cluster, 15 berries were taken and 225 berries of each replicate were subjected to berry width (mm), berry length (mm), berry weight (g), berry volume (ml) analyses. The must obtained through squeezing the berries were subjected to total soluble solids content (TSSC) (%), pH, acidity (%) and ripening index (TSSC/Acidity). Number of seeds per berry (n/berry), seed weight (mg/berry) and single seed weight (mg/seed) were also determined.

Descriptive statistics for investigated parameters were presented in means  $\pm$  standard errors. Cultivars were compared with one-way analysis of variance. Statistical calculations were performed with SPSS software.

### **RESULTS AND DISCUSSION**

Results on cluster parameters of the wine grape cultivars are provided in Table 1. The greatest cluster weight, cluster volume, cluster size and cluster length values were observed in Kabarcık cultivar and the lowest values were obtained from Kalecik Karası cultivar. Cluster weights of the cultivars were ordered as Kabarcık (363.88 g), Öküzgözü (335.79 g), Hasandede (277.33 g) and Kalecik Karası (166.99 g). Cluster and berry sizes may vary from one cultivar to another (Deryaoğlu and Canbaş, 2003; Tangolar et al., 2005).

Cultivar	Cluster Width (cm)	Cluster Length (cm)	Cluster Weight (g)	Cluster Volume (ml)	Cluster Size (cm <sup>2</sup> )
Öküzgözü	10.23+2.15 ab	21.39+2.68 ab	335.79+7.12 b	302.29+8.14 b	208.13+7.56 b
Kalecik Karası	7.86+1.30 b	13.50+1.87 c	166.99+5.45 d	153.15+5.59 d	110.17+5.96 d
Hasandede	9.41+1.65 ab	18.66+1.71 b	277.33+8.12 c	250.64+8.72 с	174.20+6.14 c
Kabarcık	10.62+1.73 a	22.70+1.50 a	363.88+8.36 a	327.11+9.12 a	224.43+7.42 a
LSD % 1	0.47	1.80	30.47	29.80	28.21

Table 1. Cluster Parameters of Wine Grape Cultivars

The differences between means indicated with different small letters in the same column are significant (P<0.01), N.S.: Not Significant.

Berry size is a significant factor for the quality of wine grapes (Mattews and Nuzzo, 2007). It was reported in previous studies that berry size altered fruit composition (tannin, anthocyanin) and greater tannin and anthocyanin concentrations were observed in wines obtained from small berries (Mattews and Nuzzo, 2007). The greatest berry size parameters (berry width, berry length, berry weight and berry volume) were observed in Öküzgözü and Kabarcık cultivars and the smallest values were observed in Hasandede cultivar (Table 2). Present findings were similar with the berry weights reported by Uzun and Bayır (2008) for Kalecik Karası cultivar.

Cultivar	Berry	Berry	Berry	Berry
Cultival	Width (mm)	Length (mm)	Weight (g)	Volume (ml)
Öküzgözü	16.95±0.47 a	17.90±0.24 a	3.46±0.34 a	3.20±0.15 a
Kalecik Karası	13.44±0.34 b	14.04±0.17 b	1.98±0.21 ab	1.84±0.08 a
Hasandede	12.06±0.38 b	12.52±0.29 c	1.40±0.09 b	1.30±0.12 ab
Kabarcık	15.67±0.27 a	16.49±0.15 a	2.92±0.16 a	2.70±0.06 b
LSD %1	0.38	0.41	0.21	0.19

Table 2. Berry Parameters of Wine Grape Cultivars	Table 2.	Berry	Parameters	of Wine	Grape	Cultivars
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The differences between means indicated with different small letters in the same column are significant (P<0.01), N.S.: Not Significant.

Quality of grape cultivars largely depends on berry composition. Berry composition is dominated by TSSC, organic acids, pH and phenolics. These attributes may vary with the genetic structure of the cultivars, vineyard location, altitude, orientation, climate factors, rootstocks, cultural practices and harvest time (Karanis and Celik, 2002). In wine cultivars, TSSC criterion for harvest is generally indicated as 19.0-23.0% for white cultivars and as 20.5-23.5% for red cultivars (Rieger, 2006). Wine alcohol levels increase to certain levels with the increasing TSSC of the berries (Cox, 1999). The greatest TSSC was observed in Kalecik Karası (22.1%) and the lowest value was seen in Kabarcık (19.85%) cultivar (Table 3). Gök Tangolar et al. (2009) in a study carried out under Adana conditions, reported TSSC of Kalecik Karası cultivar as 25.5%. In present study, TSSC of Kalecik Karası cultivar was identified as 22.8%. Titratable acidity is used as a measure of total acids in grape juice and expressed as tartaric acid equivalent. In general, acidity is recommended to be between 0.65-0.85% for white wine cultivars and between 0.60-0.80% for red wine cultivars (Cox, 1999). Grape acids inhibit microorganism development in must. The greatest acidity was observed in Hasandede (0.72%) and the lowest acidity was seen in Kabarcık (0.46%) cultivar (Table 3). The pH should not exceed 3.3 in white wine cultivars and 3.5 in red wine cultivars (Cox, 1999). High fruit juice pH levels reduce wine quality (color, taste, and etc.) (Kodur et al., 2010). Fruit juices with high pH result in wine defects by destructing organisms. Increase in pH goes on during the ripening process and play a distinctive role in identification of harvest time (Karanis and Çelik, 2002). The optimum pH levels were indicated as 3.1 or 3.2 for white wine cultivars and 3.4 for red wine cultivars (Cox, 1999). Among the investigated cultivars, the greatest pH was observed in Kabarcık (3.28) and the lowest pH was observed in Hasandede (3.18) cultivar (Table 3). Tangolar et al. (2002) reported that pH of the cultivars varied with the years and reported the pH values of the 2000 year as 3.15 for Carignane; 3.37 for Chardonnay; 3.30 for Narince, 3.45 for Kalecik Karası and Semillon. In another study, Tangolar et al. (2005) reported fruit juice pH values as between 2.94-3.06 for Carignane, between 2.90-2.93 for Kalecik Karası and between 2.93-3.53 for Semillon cultivar. Present pH values comply with the ones reported by Özden and Vardin (2009) and Cangi et al. (2009) for Chardonnay grape cultivar. The pH values of the other cultivars comply with the pH values reported by Tangolar et al. (2002).

Cultivar	TSSC (%)	Acidity (%)	РН	Ripening Index
Öküzgözü	21.1±0.45 ab	0.65±0.04 b	$3.26 \pm 0.03$	32.46±2.14 a
Kalecik Karası	22.8±0.83 a	0.53±0.02 c	$3.24 \pm 0.02$	43.01±3.45 a
Hasandede	20.7±0.35 ab	0.72±0.03 a	$3,18{\pm}0.04$	28.72±1.90 b
Kabarcık	19.8±0.62 c	0.46±0.02 d	$3.28 \pm 0.05$	43.04±4.12 a
LSD %1	1.2	0.06	N.S.	5.8

Table 3. Fruit Juice Quality Parameters of Wine Grape Cultivars

The differences between means indicated with different small letters in the same column are significant (P<0.01), N.S.: Not Significant.

Seed of wine grape cultivars are used as waste material for various purposes. Grape seeds contain about 60-70% polyphenols and have significant impacts on human health (Uzun and Bayır, 2008). Grape seeds are sold as a drug in Europe. Proanthocyanidine is the strongest antioxidant discovered in nature of the seeds. Antioxidants serve a defense mechanism against free radicals (Özcan et al., 2008). It is important for human health that seeded table grapes should be used and waste seeds of wine production processes should be used for different purposes. Therefore, seed parameters of the wine grape cultivars were also investigated in present study. The greatest single seed weight was observed in Öküzgözü (49,24 mg) and the lowest values were observed in Kalecik Karası (36.31 mg) and Kabarcık (34.65 mg) cultivars. The differences in number of seeds per berry of the cultivars were not found to be significant (Table 4). Deryaoğlu and Canbaş (2003) indicated that grapes generally have two seeds, but the number could vary from berry to berry of the same cluster.

Cultivar	Seed Weight (mg/seed)	Number of Seed (n/berry)	Single Seed Weight (mg/berry)
Öküzgözü	49.24±2.14 a	$2.96 \pm 0.09$	96.60±4.27 a
Kalecik Karası	36.31±1.16 c	2.44±0.11	68.98±3.55 c
Hasandede	42.37±1.92 b	2.69±0.10	82.01±2.86 b
Kabarcık	34.65±0.95 c	$2.37 \pm 0.08$	65.61±3.22 c
LSD%1	1.8	<b>N.S.</b>	6.73

Table 4. Seed Parameters	of Wine Gra	pe Cultivars
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The differences between means indicated with different small letters in the same column are significant (P<0.01), N.S.: Not Significant.

Correlation analysis was performed to determine the relationships between the investigated parameters and results are provided in Table 5. There were significant positive correlations (r=0.78-0.86) between cluster weight and berry parameters (width, length, weight, volume). The correlations between cluster size, cluster volume and must parameters (pH, TSSC, acidity, TSSC/acidity) were not found to be significant. There were significant (p < 0.01) negative correlations between berry parameters (berry width, berry length, berry weight and berry volume) and pH (r=-0.45 - 0.59) and TSSC (r=-0.45--0.59). Matthews and Nuzzo (2007) also reported significant negative correlations between TSSC and berry size. Berry parameters positively correlated with total seed weight of the berry (r=0.41-0.53) and berry width at 5% level and with the other berry parameters at 1% level. Barbagallo et al. (2011) reported correlations between berry weight and berry seed weights of Syrah cultivar. Karanis and Çelik (2002) reported increasing pH value with increasing TSSC levels. Significant positive correlations were also observed in this study between pH and TSSC (r= 0.70). The pH also positively correlated with ripening index (r = 0.78) and negatively correlated with acidity (r = -0.78). There were negative correlations between TSSC and acidity (r = -0.54) and between acidity and ripening index (r=-0.82). Present correlations well comply with the correlations reported by Kamiloğlu and Üstün, (2014). Must parameters (TSSC, pH, acidity, ripening index) generally did not have any correlations with number of seeds and berry weights. It was concluded based on present findings on some cluster and berry parameters that these cultivars could be grown in the region and with regard to fruit juice quality attributes, the Öküzgözü, Kalecik Karası and Hasandede cultivars should be preferred in the region. With regard to investigated parameters, berry size positively correlated with seed weights of the berries and negatively correlated with TSS.

	Cluster	Cluster	Cluster	Cluster	Downer	Dowwy	Down	Downy
	Cluster length	Cluster weight	Cluster volume	Cluster size	Berry width	Berry length	Berry weight	Berry volume
	(cm)	(g)	(ml)	(cm <sup>2</sup> )	(mm)	(mm)	(g)	(ml)
Cluster width	0,78**	0.81**	0.83**	0.96**	0.63**	0.63**	0.69**	0.69**
Cluster length (cm)		0.93**	0.85**	0.97**	0.74**	0.83**	0.74**	0.74**
Cluster weight (g)			0.98**	0.90**	0.78**	0.86**	0.78**	0.78**
Cluster volume (ml)				0.95**	0.71**	0.79**	0.82**	0.82**
Cluster size (cm <sup>2</sup> )					0.79**	0.79**	0.83**	0.83**
Berry width (mm)						0.89**	0.89**	0.89**
Berry length (mm)							0.96**	0.91**
Berry weight (g)								0.96**
	pН	TSSC	Acidity	Ripenin	g Seed weigl		ea	ingle seed weight
	-	(%)	(%)	index	(mg		erry)	(mg/t berry)
Cluster width	-0.12(1)	-0.25(1)	-0.25(1)	$0.17^{(1)}$	) 0.54	** -0.	.26 <sup>(1)</sup>	0.59**
Cluster length (cm)	-0.28(1)	-0.17(1)	-0.10 <sup>(1)</sup>	-0.07(1	0.57	** -0.	.26 <sup>(1)</sup>	0.62**
Cluster weight (g)	-0.45*	-0.31(1)	-0.12(1)	-0.07(1	0.67	** -0.	.36 <sup>(1)</sup>	0.50**
Cluster volume (ml)	-0.26 <sup>(1)</sup>	-0.29(1)	-0.19 <sup>(1)</sup>	-0.10(1	0.64	** -0.	.26 <sup>(1)</sup>	0.55**
Cluster size (cm <sup>2</sup> )	$-0.22^{(1)}$	-0.25(1)	-0.19 <sup>(1)</sup>	-0.03(1	0.60	** -0.	.26(1)	0.65**
Berry width (mm)	-0.45*	-0.45*	-0.10 <sup>(1)</sup>		0.60	** -0	.44*	0.41*
Berry length (mm)	-0.59**	-0.59**	$0.25^{(1)}$	-0.30(1	0.61	** -0	.44*	0.50**
Berry weight (g)	-0.55**	-0.50**	$0.10^{(1)}$	-0.30(1	0.58	** -0	.45*	0.51**
Berry volume (ml)	-0.56**	-0.50**	0.09(1)	-0.30(1	0.58	** -0	.40*	0.53**
рН		0.70**	-0.78**	0.78*	* 0.20	<sup>(1)</sup> 0.	35(1)	0.09(1)
TSSC (%)			-0.54*	0.78*	* 0.34	<sup>(1)</sup> 0.	35(1)	$0.05^{(1)}$
Acidity (%)				-0.82*			.20(1)	-0.42*
Ripening Index					-0.17		.18(1)	$0.29^{(1)}$
Seed weight (mg)							).70	0.36(1)
Seed number n/berry	,							0.19(1)
**. Cignificant com	lation (m	-0.01). *.	Cianifiaan	+	tion (m/	(1)	Not Cia	ificant

Table 5. Correlation	Coefficients Between	n Investigated Q	Quality Traits in	Cultivars

\*\*: Significant correlation (p<0.01); \*: Significant correlation (p≤0.05); <sup>(1)</sup>: Not Significant.

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## **CHAPTER 10**

## **COLD STRESS IN PLANTS**

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### **INTRODUCTION**

Plants have an extremely important place in the life of living things and in the ecological cycle. They primarily undertake complex events such as photosynthesis, while also undertaking critical tasks of preventing erosion, contributing organic matter to the soil, providing temperature control, and becoming raw material for different industrial areas. It is also known that the ecologies of plants that have such an important place in our lives change day by day and face numerous negative factors. Plants that are an integral part of the ecosystem are unable to change the place where they are so they are trying to sustain their growth and development by having variable responses depending on their genetic adaptation to stressors (Wolters & Jurgens, 2009; Golldack et al., 2011).

In plants, the stress condition occurs in two ways, biotic and abiotic. Biotic stress is the adverse conditions that occur when the creature encounters another creature (insect, fungus, bacteria, etc.). Abiotic stress, on the other hand, is stress caused by all complex environmental conditions such as drought, salinity, strong and low light, ultraviolet light, high and low temperature, freezing, heavy metals, and insufficient oxygen. Abiotic stresses threaten the future of agricultural production by reducing yields in agricultural production by about 50% (Mahajan & Tuteja, 2005; Vij & Tiyagi, 2007).

Low temperature/cold is a decisive factor that limits the development of many plant species and is effective for the normal distribution of plants on Earth (Jeon & Kim, 2013; Chen et al., 2014). About 25% of the terrestrial area on Earth consists of regions that do not fall below 15 °C and are reliable for frost damage. In the rest of the region, particularly sensitive plants can be harmed by dropping temperatures below 0 °C during certain periods (Puhakainen, 2004). The temperature of plant tissues varies depending on the ambient temperature. So they have to regulate their metabolism depending on the ambient temperature. In this process, high plants try to adapt to cold by displaying varying levels of changes such as physiological modifications, cell membrane compositions, and protein and metabolite content (Kazemi-Shahandashti & Maali-Amiri, 2018). Because of these complex metabolism activities, cold stress is extensively addressed by investigators. In this regard, studies of different plants related to classical breeding or molecular techniques have been carried out and continue to be carried out to ensure cold resistance in plants.

In this section prepared as a review study, the effects of low temperature on plants and the mechanisms of action that provide the resistance in resistant plants are tried to be expressed.

# 1. CLASSIFICATION OF PLANTS ACCORDING TO COLD STRESS

In general terms, cold damage means that the water inside the cell expands by the cold effect and, the plant dies as a result of breaking down the cell walls due to freezing. In fact, because ice crystals, which form as a result of freezing in plant tissues, take shape in extracellular areas due to dehydration, the plant cell contracts at low temperatures. In the natural process, the formation of ice crystals within the living cells of a frozen plant is not observed. While it is known that freezing water outside the cell damages the membranes during melting, it is also known that this damage does not lead to the complete breakdown of the membrane and the destruction of the cell wall.

Overall, an optimum temperature degree is required for the normal growth and development of each plant. Temperatures below this degree are perceived as cold stress in plants. Cold and freezing stresses are different stresses. Cold stress has a direct effect on cells. Freezing plays a role indirectly, damaging cells with ice formation.

There are differences between plant tissues seasonally in resistance to cold, making the explanation of the cold resistance mechanism more complex. In Central European pines, temperatures of -10 °C in the summer period cause the death of leaves, while in winter they remain vibrant even at much lower degrees (Grene, 2002; Beck et al. 2004). It is thought that there will be significant increases in the overall yields when resistance to a 2 °C frost can be achieved in citrus fruits, winter grains, potatoes, deciduous fruit trees, and some vegetables. In the opposite case, significant losses may emerge. 1 °C decrease in the Earth's average temperature could lead to a 40% reduction in rice production (Pearce, 1999).

According to the exposure to cold stress, plants are divided into 3 groups (Turan & Ekmekçi, 2008; Doğru, 2019):

- Cold-sensitive plants (plants damaged by temperatures below 12 °C); Low temperature-sensitive tropical plants such as bananas (*Musa sapientum*), avocados (*Persea spp.*), and papaya (*Carica papaya*) are included in this group.

- Cold-tolerant but frost-sensitive plants (plants that can adapt to temperatures below 12 °C but do not survive freezing temperatures); Plants of subtropical origin, such as potatoes (*Solanum tuberosum*) and tomatoes (*Solanum lycopersicum*), are included in this group. These plants are low temperature-resistant but freezing-sensitive.

- Plants with a tolerance for freezing (plants that can survive and adapt to below-freezing temperatures); They are plants that can adapt to the cold and live in subzero temperatures as well. Plants of temperate climate origin are included in this group.

### 2. DETECTION OF COLD STRESS IN PLANTS

Plants' response to environmental stress occurs through a series of reactions called signal transmission. The low-temperature signal transmission also begins again with the detection of the cold signal by a receptor. With the perception of cold stress, the cytoskeleton is rearranged. In this process, the activation of  $Ca^{+2}$  channels, the increased levels of  $Ca^{+2}$  in the cytosol, and the triggering of the expression of cold-regulated genes (COR) (Chinnusamy et al., 2006) occur. Abscisic acid (ABA) constitutes an effective chemical message for adapting to stress (Ton & Flors, 2009; Xue Xuan et al., 2010). It is known that ABA synthesis is related to cold resistance (Shi & Yang, 2014), mutant plants that cannot synthesize ABA have less ability to adapt to cold (Liorente et al., 2000), and ABA has an effect on the expression of certain genes.

### 2.1. Proteins that detect the role and signaling of calcium

One of the earliest responses a plant gives to low temperature is that the amount of free  $Ca^{+2}$  in the cytosol temporarily rises. A cold-induced  $Ca^{+2}$  acts as a second messenger against cold stress (Chinnusamy et al., 2010). Signals are distributed through  $Ca^{+2}$  and special calcium-bound proteins such as Calmodulin (CaM) are activated (Solanke & Sharma, 2008). CaM is one of the best preserved  $Ca^{+2}$  bound proteins in eukaryotes. CaM activates a large number of target proteins involved in various cellular functions in  $Ca^{+2}$  binding (Snedden & Fromm, 2001).

Calcium-dependent protein kinases (CDPKs) act as important sensors in the response to abiotic stresses, which also include cold stress. Mitogenactivated protein kinases (MAPKs) are serine/threonine protein kinases that play a key role in complementing multiple intracellular signals transmitted by various secondary messengers. MAPK is involved in signal detection in all eukaryotic cells during the response to extracellular stimulants. MAPK is also the sensor of the  $Ca^{+2}$  signal in the cold (Jonak et al., 1999; Turan & Ekmekci, 2008).

## 2.2. Regulation of gene expressions

Many studies have been carried out to reveal the isolation, and characterization of cold-sensitive genes and genetics of cold acclimation in different plant species and varieties. The synthesis of cold-inducing proteins occurs with variations in the translation of mRNA. Studies have established an association between change in mRNA profile and increased endurance to frost. To date, a large number of stimulated genes have been obtained in many plants by examining cDNA libraries of plants that are accustomed and unaccustomed to cold. Nucleotide sequences of these cDNA clones have been determined. Most of the studies were conducted on wheat and other grains. The inheritance of frost resistance in wheat and woody plants is controlled by multiple genes. The cumulative effects of both dominant and recessive genes have also been determined. Although the 5A and 5D chromosomes in wheat showed substantial effects on frost resistance, 11 in 21 chromosomes were determined to be effective at resisting frost (Thomashow, 1990).

It has been reported that cold adaptation is regulated by increasing or decreasing the expression of hundreds of genes (Fowler & Thomashow, 2002). COR proteins, which are key in adapting to cold, protect cells against cold stress, help with the expression of other proteins, and aid in the formation of necessary biochemical changes (Theocharis et al., 2012). Some COR genes also include ABA-response elements. The expression of COR genes is regulated in both ABA-dependent and ABA-independent metabolic ways (Shinozaki & Yamaguchi-Shinozaki, 2000).

Frost resistance mechanism-related genes in plants have been studied separately in the form of low-temperature response element (LTRE), cold-responsive genes (COR), and ABA-responsive genes (ABRE) (Puhakainen, 2004). The ABA is related to the process of cold adaptation in plants. It was determined that the ABA increased in herbaceous and woody plants exposed to low temperature and its external application led to increased chill and frost tolerance. Some mutant plants with low ABA content have also been found to be damaged in the cold adaptation process (Smallwood & Bowles, 2002; Puhakainen, 2004).

Analysis of gene expressions, which can be induced by cold during low temperature, shows that at least two groups exist whose expressions occur at different times. In the first group, in response to low temperature, the expression is swift and fleeting. The second group contains delayed response genes. Expressions of these genes, including COR genes, gradually increase during cold application and are sustained for a long time (Mahajan & Tuteja, 2005).

## 2.3. Dehydrins

Dehydrin proteins are produced in the process of responding to environmental stimulants with dehydrative elements in plant cells, in developmental phases such as seed and pollen maturation (Svensson et al., 2002). Dehydrins are found in all photosynthetic organisms. They are induced by cold and dehydration, their induction is related to the type of organ and depends on the development phase of the tissue (Rorat et al., 2004). Dehydrins settle in the nucleus, cytoplasm, mitochondria, vacuole, and around the cell membrane (Heyen et al., 2002; Borovskii et al., 2005). The most accumulation of many dehydrins has been observed in vascular tissues, which are more sensitive to low-temperature damage, and in the cells surrounding these tissues (Nylander et al., 2001). Dehydrins are known to have the effect of stabilizing the cell structure against dehydration (Danyluk, et al., 1998), and cold-protecting (Bravo et al., 2003; Sanchez-Ballesta et al., 2004), antifreeze (Wisniewski et al., 1999) and osmoregulator (Nylander et al., 2001).

## **3. EFFECTS OF COLD STRESS IN PLANTS**

Plants develop various strategies to be able to tolerate the cold stresses they are exposed to. However, while these strategies are effective up to certain temperatures, but not sufficient when exposed to extremely low temperatures, thus some damages occur to plants.

The resistance mechanism to cold stress in plants is a complex condition in which many factors act. Therefore, many important factors need to be addressed to uncover this. Firstly, the mechanism of intracellular and intercellular frost formation, the changes in moisture and dry matter content in tissue prior to and during the formation of frost, and changes in lipids, protein, certain amino acids, antioxidant enzymes, macro, and micronutrient elements need to be examined.

Cold damage at low temperatures below freezing is physical and/or physiological changes triggered by the plant's exposure to the cold. Physiological changes can be divided into primary and secondary damage. The primary damage is the first rapid response that causes dysfunction in the plant, which can easily recover as a result of the temperature rising to normal conditions. Secondary damage, on the other hand, is dysfunction as a result of primary damage and may not be reversible. Characteristic visuals of cold damage are caused by secondary cold damage (Rab & Saltveit, 1996).

## 3.1. Effects of cold stress at the cellular level

The first region where freezing harms occur in plant cells is the membranes. Hence the changes in cellular membranes throughout the adaptation to the cold are quite significant.

The cell membrane contains a characteristically high proportion of phospholipid, free and glycosylated sterol, and cerebrosides. A number of changes occur in the lipid content of the cell membrane during contact with the cold. First, in common with many species, an increase in the phospholipid rate of the cell membrane is observed, and the ratio of cerebrosides in the cell membrane decreases at the level of maximum tolerance to cold in increasing cold stress. This decrease is also varied by plant species (Uemura & Steponkus, 1999).

In the cellular membranes of cold-sensitive plants, there are more saturated fatty acids. These types of membranes tend to solidify at low temperatures. This phase change prevents the proteins in the membranes' structure from performing their normal function. In resistant plants, the activities of desaturase enzymes and therefore the amount of unsaturated fatty acids in the structure of the membranes increase during the adaptation to the cold. When the duration of cold stress suffered by the plant is short, the adverse effects can be repaired. Prolonged stress causes one after the other harm such as lack of energy metabolism, deterioration of photosystems, cell autolysis, and death (Turan & Ekmekçi, 2008).

# **3.2.** The effects of cold stress on organs at different plant development stages

Naturally, the periods in which plants are harmed by cold are extremely important. The organs most affected by the cold in fruit trees are flower buds. These are also very susceptible to cold, especially during periods when they start blooming. The resistance of flower buds to cold also varies, again, according to the development period. Plants are susceptible to cold stress during the process of the formation of reproductive organ, flowering, pollen production, pollen germination, and seed formation. Pollen germination and pollen tube formation are suppressed at low temperatures (Clarke & Siddique, 2004). Any disorder caused by cold stress in the male or female parts of the flower causes the shedding of flowers (Nayyar et al., 2005). Infertility can occur in the event of low-temperature exposure during the blooming period.

The seed is very tolerant of prolonged low temperatures due to its low humidity content and being dormant. With water intake, it can become susceptible to cold. Cold delays the absorption of water or disrupts the membrane integrity during its initial absorption, increasing electrolyte leakage and preventing germination (Bois et al., 2006). The seed germination phase and early seedling developmental phases are highly affected by low temperatures (Adhikari et al., 2021). Growth and development are suppressed, and even the apical bud is damaged in hypersensitive species (Prasad et al., 2006). In the vegetative phase, seedlings are often more sensitive to cold than mature plants. Exposure of cold-sensitive seedlings to low temperatures above freezing results in reduced root transmission and water intake so reduced root growth (Aroca et al., 2001).

The major morphological symptoms of cold stress are chlorosis, reduction in trunk elongation, surface lesions in some parts, leaf curlings, discoloration, and tissue damage (Goering et al., 2021). Fracture and cracking in the trunk, metabolite leakage, leaf yellowing and necrosis occur, thus growth is inhibited (Adhikari et al., 2021).

### 3.3. Effects of cold stress on photosynthesis

Low temperatures slow down all metabolic reactions. However, there are two sensitive metabolic reactions, especially in terms of photosynthesis; these are reactions that regulate  $CO_2$  fixation and stomata opening. Therefore, inhibiting the stomata's ability to regulate dehydration and the change of  $CO_2$  are two major consequences of leaves' exposure to cold. Stomata openings usually decrease at low temperatures (Vernieri et al., 2001). Reduced stomata openings, as well as reduced water permeability of the roots, have a negative effect on photosynthesis due to changing water relationships (Yang et al., 2005).

# 4. FACTORS AFFECTING COLD ADAPTATION AND COLD RESISTANCE

Various plant species have the ability to increase their cold or freezing tolerance by exposure to low but not freezing temperatures for a period of time. This event is known as "cold adaptation or cold acclimation" (Thomashow, 1999).

Plants can be more resistant to cold by undergoing natural acclimation during the fall period (Wisniewski et al., 2003). Many events are required to gain maximum adaptation in the process of acclimating to cold. These can be indicated as hormonal changes along with environmental changes, altered gene activity, and new gene products, changes in the accumulation of soluble substances and lipid composition (Howarth & Ougham, 1993).

In the event of excessive cooling of water in tissues, the accumulation of antifreeze material can be effective in protecting against frost stress (Aslantaş et al., 2010). The level of cold tolerance achieved by cold adaptation is not static. It can vary depending on the seasons and is quickly lost when returned to the temperature where adaptation is not required. But plants with these gene expressions gain tolerance by making these changes during adaptation. In a successful cold adaptation, the plant's photosynthetic activity at low temperatures, especially in moderate and high light conditions, must be well adjusted (Wanner & Junttila, 1999). Cold adaptability is a polygenic property that involves numerous genes and where these gene expressions are mostly controlled at low temperatures. Changes in the expression levels of genes in the cold adaptation process cause many molecular and physiological changes (Krebs et al., 2002).

## 4.1. Metabolite accumulation

The most common behaviors of plants to resist cold stress are the accumulation of cryoprotective polypeptides such as soluble sugar and proline (Ritonga & Chen, 2020). *Euphorbia resinifera, Echinocactus grusonii, Aloe vera, Crassula lacteal, Bryophyllum pinnatum, Yucca aloifolia,* and *Sansevieria trifasciata* have been determined to increase sugar accumulation after cold stress (Khan et al., 2015). Plants adapted to the cold always accumulate more sugar (D-Glucose, D-Glucose 6 Phosphate, amylose, starch, and maltose) in the tissues under the soil (Londo et al., 2018).

Proline is deposited in the cytosol in response to stresses in plants. As an osmoregulator, it contributes to tolerance for the cold. Proline protects cell membranes and proteins from the harm of low temperature and high concentration of inorganic ions (Atici et al., 2003).

Increasing the rate of unsaturated membrane lipids during cold adaptation is crucial to sustaining membrane fluidity (Uemura et al., 2006)

### 4.2. Antioxidant defense mechanisms

In low-temperature conditions, reactive oxygen species (ROS) production is increased excessively in susceptible plants, and simultaneously antioxidant enzyme activities such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) are inhibited (Alam & Jacob, 2002; Takac, 2004). Antioxidant defense systems needed for detoxification of harmful ROS are found in all plant cells (Seppänen & Fagerstedt, 2000). These antioxidant defense systems are classified as enzymatic and non-enzymatic (Hernández-Nistal et al., 2002).

Enzymatic defense systems consist of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR). Non-enzymatic defense systems consist of ascorbate, glutathione, carotenoids,  $\alpha$ -tocopherol, anthocyanins, and flavonoids (Choi et al., 2002).

### 4.2.1. Enzymatic defense systems

Superoxide dismutase plays an important role in the survival of the plant in conditions of oxidative stress caused by biotic and abiotic stresses. There are several types according to whether it contains copper and zinc (Cu/ZnSOD), manganese (MnSOD), or iron (FeSOD) in its active regions. Cu/ZnSOD is found in the cytosol and chloroplast of the plant cell. MnSOD is found in mitochondria and peroxisomes. The SOD enzyme converts  $O_2^{\bullet-}$  to  $H_2O_2$  (Kireçci, 2018). It is necessary to remove the resulting  $H_2O_2$  from the environment immediately so that it does not damage the cell. This is because  $H_2O_2$  forms a highly reactive OH radical under certain conditions. The detoxification of  $H_2O_2$ , which is formed as a result of the reaction catalyzed by SOD, can be achieved by three different enzymes: POD, CAT, and APX.

POD converts  $H_2O_2$  into the water through the oxidation of metabolites such as phenolic compounds and/or antioxidants (Sudhakar et al., 2001; Morsy et al., 2007). CAT in peroxisomes eliminates  $H_2O_2$  that comes to peroxisomes by diffusion from the cytosol (Lukatkin, 2002). APX is the first enzyme of the ascorbate glutathione cycle, which is found in mitochondria, chloroplasts, cytosols, apoplast, and peroxisomes, reducing  $H_2O_2$  to water. The high affinity of APX to  $H_2O_2$  and the presence of the ascorbate glutathione cycle in almost every cellular structure indicate that this cycle is vital in controlling the level of ROS.

## 4.2.2. Non-enzymatic defense systems

Non-enzymatic defense systems such as ascorbic acid, glutathione,  $\alpha$ -tocopherol, carotenoids, and flavonoids also play important roles in rendering ROS harmless.

Ascorbate is synthesized in mitochondria and transported to other cell components through a proton-electrochemical mechanism or facilitated diffusion (Horemans et al., 2000). Ascorbic acid acts on many physiological processes that regulate the growth, differentiation, and metabolism of plants (Smirnoff, 2011). Ascorbic acid acts as a reducing agent and removes many types of free radicals and provides membrane protection by recovering tocopherol from the tocopheroxyl radical (Kireçci, 2018).

Glutathione is a metabolite abundant in plants and has numerous and important functions (Foyer & Noctor, 2005). Glutathione, a tripeptide detected in almost all cell divisions such as cytosol, chloroplasts, endoplasmic reticulum, vacuoles, and mitochondria, is an antioxidant that acts in various ways. In the ascorbate-glutathione cycle, GSH is used to reduce both enzymatic and non-enzymatic DHA (dehydroascorbate), and it is oxidized to GSSG (oxidized glutathione). The central role of GSH in the antioxidant defense is due to its ability to regenerate ascorbic acid through the ascorbate-glutathione cycle (Millar et al., 2003).

Tocopherols are antioxidants found in all parts of plants and algae (Srivalli et al., 2003) and are composed of four isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ). Among them,  $\alpha$ -tocopherols are the most biologically active and dominant antioxidants in chloroplast membranes. It has a protective effect against photo-oxidative damage. Its antioxidant properties depend on its ability to bind the singular oxygen. The most important function of tocopherols is that they participate in various mechanisms related to polyunsaturated fatty acids (Ledford et al., 2007). Also,  $\alpha$ -tocopherol levels increase in photosynthetic plant tissues in response to various abiotic stresses (Noctor, 2006).

Flavonoids are various secondary metabolites that are abundant in plant tissues. Polyphenols have an ideal structure for free radical scavenging activity. It has been shown that there are more effective antioxidants than tocopherol and ascorbate (Kireçci, 2018). The antioxidant properties of polyphenols are due to their high reactivity as hydrogen or electron donors

and their ability to stabilize the polyphenol-derived radical. They have the ability to chelate the unpaired electron and transition metal ions (Rice-Evans et al., 1997). Anthocyanins are one of the flavonoid compounds synthesized in plants through the metabolism of shikimic acid, which uses phenylalanine as a substrate. When plant stems are exposed to cold, they accumulate anthocyanin in the vacuoles of epidermal and sub-epidermal cells and thus their color turns red (Leng et al., 2000).

In addition to the growth and development events they undertake in the plant, plant hormones also act as a signaling molecules. Cytokinins, auxins, gibberellins, ethylene, salicylic acid and brassinosteroids, jasmonic acid, and strigolactones, as well as ABA, are also effective in signaling cold stress and transcription of some COR genes (Deng et al., 2018). Under cold stress, the level of ABA and jasmonic acid in plants increases, and the level of cytokinin, ethylene, and gibberellin decreases. Plant hormones are also ROS-binding and perform tasks related to the metabolism of biomolecules such as sugar and starch (An et al., 2012).

#### 4.3. Plant nutrient elements

As a result of freezing/melting stress, membrane permeability is lost along with ion loss in cells. The basic cation that disappears from cells with frost damage is potassium (K<sup>+</sup>). Since the transport of K<sup>+</sup> through the cell plasma membrane is by the activity of H+ -ATPase, it is noted that there is a change in the function of H+ -ATPase during freezing/melting stress. In order for the tissues to recover after freezing/melting damage, the lost ions must be pumped back into the cells (Iswari & Palta, 1989). In the studies, it was determined that the administration of Sodium Vanadate, an inhibitor of H+ -ATPase, prevents the recovery of tissues as a result of frost stress (Arora & Palta, 1991).

### **5. CONCLUSION**

Cold stress is one of the important stress factors that have a restrictive effect on plants. It can cause oxidative stress, catabolism, and decreased photosynthesis rate in susceptible plants and cause the death of plants in the advanced stage. It is a well-known fact that cold stress is almost impossible to control compared to other stress factors. However, given the rapidly growing world population and, on the contrary, the decreasing agricultural lands, it is imperative to prevent losses in plants that are necessary for human nutrition. The steps that may be taken to combat stress are crucial in this approach. Therefore, it is necessary to clarify the physiological and biochemical mechanisms that ensure cold tolerance and adaptation in plants, to develop cold-tolerant plants, and thus increase crop yields.

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# **CHAPTER 11**

## THE ROLE OF BACTERIAL EXOPOLYSACCHARIDES ON THE AMELIORATION OF SALT STRESS IN PLANTS

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### **INTRODUCTION**

Our world is facing severe environmental stresses because of aggravated human activities and rapidly changing climate conditions, which plays an adverse role in agricultural production (Sunita, et al., 2020). Plant growth and development are negatively affected by several factors such as salinity, nutrient limitation, droughts, temperature, soil-borne pathogens, heavy metals etc., therefore raising serious concern for global food security. The reduced agricultural lands due to urbanization and a speedily growing human population also increase the demand for food, thus crop quantity and quality should be guaranteed for a better future.

Excess amounts of soluble salts in soils negatively affect the growth and development of a broad range of plants globally, thus being considered among the most harmful threats to food security. Most parts of the world's land face salinity-induced problems, which causes negative impacts on economy, environment, and social life. Srivastava et al. (2019) claimed that salty soils negatively influence people's livelihoods in over 100 countries. The effect of salinity on the agricultural economy exceeds 27 billion US\$ annually due to the losses in crop production (Qadir, et al., 2014). The yield loss might be up to 80% (Zörb, et al., 2019), thus effective strategies should be implemented to cope with the detrimental effects of salinity in plants. Furthermore, it was estimated that by 2050, salinity will affect 50% of all arable lands (Butcher, et al., 2016) and the demand for food production will increase by ~70% due to the rapidly growing world population (Zörb, et al., 2019). The improvements in crop performance under salt stress conditions appear to be essential to ensure global food security.

Plants confer tolerance to salt-induced stress through various morphological, physiological, and biochemical mechanisms (Gupta and Huang, 2014). Certain plant growth promoting rhizobacteria (PGPR) also contribute to plant resistance to salinity in a number of protective mechanisms including antioxidant enzyme activity, activation of stress-responsive genes, the production of secondary metabolites, phytohormone synthesis, and exopolysaccharide production (Kohler, et al., 2009; Kang, et al., 2014; Bharti, et al., 2016; Sunita, et al., 2020). Among PGPR-mediated mechanisms, exopolysaccharide production by PGPR appears to be better in the amelioration of salt stress (Ashraf, et al., 2004; Ansari, et al., 2019; Sunita, et al., 2020). Under salt-stressed conditions, the accumulation of soluble salt ions increases in plant tissues, therefore causing osmotic stress and ion toxicity. Salinity also deteriorates soil stability and fertility as well as microbial interactions (Butcher,

et al., 2016). Exopolysaccharides (EPS) have a high potential to decrease the negative impacts of insoluble salts in plants by restricting the influx of salt cations into plants, increasing soil aggregation around plant roots, and stimulating biofilm formation on roots or in rhizosphere; as a result, the presence of EPS significantly improves plant growth and development under saline conditions by mitigating the effects of salinity stress through its binding potential and sticky nature.

Most of the earlier works have focused on the salt-resistant planktonic PGPR for the mitigation of saline stress. In most of those studies, the role of EPS was ignored in the enhancement of plant growth and the alleviation of salinity stress. Whereas, the best underlying mechanism of PGPR-mediated plant growth stimulation appears to be the presence of EPS and biofilm formation. Since EPS production with biofilm formation not only favors plant growth and ameliorates salt stress but also protects biofilm-forming PGPR from external stresses, EPS production along with biofilm formation seems to be more promising for mitigating saline stress and improving plant growth. Therefore, this chapter attempted to explain the underlying mechanisms of EPS along with biofilm formation in alleviating saline stress and favoring plant growth and development.

### The salinity-affected areas

Soil salinization is primarily caused as a result of the natural accumulation of soluble salts from different sources, especially in dry and semidry regions with low rainfall conditions and high evapotranspiration rate (Butcher, et al., 2016). According to Srivastava et al. (2019), around 831 million hectare-land in today's world was adversely affected by soluble salts. Of all agriculturally productive areas, around 77 million hectares have gone under salinization around the globe and salinization reduces ~1-2% of agricultural land annually (Srivastava, et al., 2019). These salinity-affected areas are continuously increasing day by day because of the excessive use of groundwater, poor irrigation practices, improper drainage systems, misuse/overuse of chemical fertilizers, prolonged waterlogging, presence of salt crystals, changing climate, and the flow of seawater into the coastal area etc. (Srivastava, et al., 2019).

### The impact of excess soluble salts on plants

Among several abiotic/natural stresses, salt stress is considered a matter of big serious concern due to its detrimental effects on agricultural production, particularly in arid and semi-arid areas (Srivastava, et al., 2019). Increasing salinity has various negative impacts on plant growth and development as well as the quality and quantity of crops. A high level of salt plays a severely harmful role in every phase of plant life from seed germination to plant maturation, thus causing severe loss in crop production (Srivastava, et al., 2019).

The inhibition of plant biological functions under saline conditions is mainly caused by osmotic stress and ion toxicity (Butcher, et al., 2016). During osmotic stress, the water retaining capacity of roots is reduced and the water loss from plants increases because of higher salinity. Osmotic stress interferes with the interactions between the root cell membrane and rhizosphere soils; thus hampering the uptake of nutrients and water by plants, reducing the growth rate of plants, causing more energy requirements, and consequently may cause cell death (Munns, 2002). Moreover, osmotic stress interrupts plant cell membrane stability, disrupts nutrient balance, and decreases photosynthetic activity and stomatal aperture (Gupta and Huang, 2014). Soluble salts disrupt water turgor pressure in the leaf by decreasing the water potential. To regulate the osmotic balance, the stomata are being closed, which lowers the transpiration rate (Srivastava, et al., 2019). As a result of stomata closure, CO2 diffusion is lessened, which inhibits photosynthesis by reducing carbon assimilation (Gupta and Huang, 2014).

Salt-stressed conditions deteriorate plant metabolisms and reduce the growth and yields of plants due to salt toxicity. Under salt stress conditions, salt ions (Na<sup>+</sup>/Cl<sup>-</sup> or Mg<sup>2+</sup>/SO4<sup>2-</sup>) highly accumulate in plants, causing ion toxicity. The higher uptake of such ions causes various physiological disorders such as ionic disequilibrium and nutritional imbalance, early leaf senescence, injured photosynthetic tissues, chlorosis, and eventually death (Zörb, et al., 2019). Under NaCl stress, uptake competition of the cation Na<sup>+</sup> with other cations and the anion Cl<sup>-</sup> with other anions results in the disturbance of ion homeostasis and nutritional balance, consequently restricting plant growth and development. As a consequence of higher salinity, excess uptake of Na<sup>+</sup> inhibits the uptake of essential ions such as K<sup>+</sup> or Ca<sup>2+</sup>, which disrupts nutritional balance and impedes crop productivity. Since the excessive uptake of Na<sup>+</sup> causes a reduction in the entry of potassium or calcium into plants, it is considered the most negative ion on plant metabolism (Butcher, et al., 2016). K<sup>+</sup> is an essential macronutrient that acts as a cofactor, maintains turgor pressure, and involves in protein synthesis; therefore, the inhibition of K<sup>+</sup> uptake (i.e. the replacement of potassium with sodium) under NaCl stress interferes with potassium-dependent biochemical and metabolic processes (Butcher, et al., 2016; Srivastava, et al., 2019). As in the case of  $K^+$  deficiency, the reduced  $Ca^{2+}$  uptake has a negative

influence on plant metabolisms. Replacement of the cation calcium with Na<sup>+</sup> results in protein denaturation and destabilization, and the prolonged existence of calcium in soil may cause drought stress in plants (Butcher, et al., 2016). For instance, previous studies observed that sodium-induced K<sup>+</sup> and Ca<sup>2+</sup> deficiency reduces shoot growth and dry weight of some certain plants, respectively (Zörb, et al., 2019). Furthermore, Cl<sup>-</sup> is an essential element for regulating enzymes and photosynthesis; however, the abundance of chloride in plants causes chlorophyll degradation, NO<sub>3</sub><sup>-</sup> deficiency, reduced photosynthetic activity, and a substantial reduction in crop yield (Butcher, et al., 2016). Excessive accumulation of Cl<sup>-</sup> might also inhibit the entry of PO<sub>4</sub><sup>3-</sup> and SO<sub>4</sub><sup>2-</sup> into plants, leading to phosphorous and sulfur deficiency, and ultimately hampering plant growth under NaCl stress (Zörb, et al., 2019).

Excessive accumulation of salts in plants causes a reduction in electron transport activity and photophosphorylation, decreasing the amount of photosynthetic pigments and carbon assimilation, impairing thylakoid membranes of chloroplast, therefore adversely affecting photosynthetic process (Srivastava, et al., 2019; Morcillo and Manzanera, 2021). Salt stress also interferes with the redox chemistry of quinone, causing an oxidative burst which disturbs photosystem II and consequently photosynthetic activity (Sharma, et al., 2012; Morcillo and Manzanera, 2021). In response to saline stress, the production of reactive oxygen species (ROS) increases, which then leads to oxidative damage to the tissues and functions of plants. Additionally, under salinity stress, abscisic acid can be increased, which in turn increases the production of hydrogen peroxide. Enhanced hydrogen peroxide production increases ROS production, leading to oxidative stress (Chinnusamy, et al., 2006; Srivastava, et al., 2019). Overproduction of ROS under salinity stress results in oxidative damage to important biological macromolecules-membrane lipids, proteins, enzymes, and DNA/RNA (Chinnusamy, et al., 2006; Sharma, et al., 2012). Salt-induced ROS burst also directly damages plant tissues, activates programmed cell suicide events, and ultimately results in cell death (Sharma, et al., 2012).

A great number of plants are not salt tolerant, thus the average yields of important crops may decline by 50-80% even at moderate salinity (Zörb, et al., 2019). This reduction represents a serious threat to agricultural production worldwide. The sensitivity/resistance of plants in response to saline stress depends on 1) the plants' ability to uptake water and nutrients from salty environments, 2) the plant growth stages, and 3) the salinity threshold level (Zörb, et al., 2019). Crop yield loss might be bigger if salinity represses plant growth during the early stages of development. For example, the negative effect of salt was more effective during the first developmental stage in cauliflower production and quality (Giuffrida, et al., 2017). Moreover, salinity tolerance depends on the salinity threshold level of plant species. The areas with EC (electrical conductivity) > 4 dS m<sup>-1</sup> are considered salinity-affected (Sunita, et al., 2020). The majority of vegetable plants exhibit a low salt threshold value (2.5 dS m<sup>-1</sup>)(Zörb, et al., 2019). According to the widely-used threshold slope model, crop productivity will be significantly lessened if the salt concentration in soil exceeds crop-specific thresholds (Butcher, et al., 2016). When the sodium concentration is above the threshold level, it interferes with plant metabolic and enzyme activity, leading to tissue necrosis and death (Kasotia, et al., 2016). Satir and Berberoglu (2016) revealed that moderate salinity (8-10 dS m<sup>-1</sup>) reduces crop yields by 55% in corn, 28% in wheat, and 15% in cotton. Among these plants, the greatest resistance was seen in cotton with a 55% yield loss at 18 dS m<sup>-1</sup> (Satir and Berberoglu, 2016).

#### The effect of salinity on microbial diversity and soil properties

In addition to the direct detrimental effects of soluble salts on biological functions of plants by the factors mentioned above, the impact of salinity on crop productivity depends on several parameters such as soil structure, microbial diversity, the water potential and nutritional conditions of soil (Butcher, et al., 2016). The existence of soil microbes is highly crucial for the cycling of nutrients essential for plant growth due to their microbial activity on the transformation of organic compounds into available nutrients through mineralization (Yan, et al., 2015). The presence of beneficial soil microbes is also vital for the maintenance of productive soil systems. Salinization degrades physical and chemical properties of soil, representing a serious threat to soil stability and fertility (Butcher, et al., 2016). Salt stress deteriorates not only soil health but also microbial activity through osmotic stress and ion toxicity, ultimately limiting crop growth and productivity. A high level of salinity causes an increment in the osmotic potential of soil, which in turn might destroy the soil microbes and root cells through plasmolysis (Yan, et al., 2015). Plants and microorganisms are able to tolerate salt stress by producing osmolytes, but the production of which requires more energy (Yan, et al., 2015).

#### Mechanisms of plant salt tolerance

Plants tolerate salinity stress through several morphological, physiological, and biochemical mechanisms, including maintaining ion

homeostasis, regulating ion transport and uptake, the production of compatible osmolytes, polyamines and nitric oxide, upregulation of antioxidant activity, and hormone regulation (Gupta and Huang, 2014). Besides plant survival mechanisms under saline conditions, certain PGPR contribute to plant salt tolerance in various ways such as enhanced antioxidant enzyme activities (Kohler, et al., 2009), regulation of stress-responsive genes (Bharti, et al., the production of secondary metabolites 2016). (volatile organic compounds/osmoprotectants/compatible solutes) (Sunita, et al., 2020), phytohormone biosynthesis and regulation (Kang, et al., 2014), and exopolysaccharide production (Ashraf, et al., 2004; Ansari, et al., 2019; Sunita, et al., 2020). Of all the protective mechanisms mentioned above, in this chapter, only the role of bacterial EPS will be addressed in the amelioration of salinity stress.

#### The roles of exopolysaccharides (EPS) on salt tolerance

During salinity stress, the concentration of specific salt ions increases in plants, disrupting the ionic equilibrium and nutritional balance of plants. To reduce the inhibitory effects of salinity stress, ion homeostasis should be maintained in plants by restricting the excess influx of salt ions by plant roots. Recent investigations have focused on the potential of exopolysaccharides in the alleviation of various stresses including salt stress (Morcillo and Manzanera, 2021).

## 1) Restricted uptake of salt ions

The first major reason how EPS mitigate salt stress is due to their cation-binding potential. The examination of the binding potential of different bacterial exopolysaccharides demonstrated that EPS have the ability to chelate cations such as Na<sup>+</sup>, thus immobilizing this type of pollutants from their surroundings (Geddie and Sutherland, 1993). In addition to Na<sup>+</sup> chelation, EPS have the potential to bind other salt cations including Ca2+, K+, and Mg2+ (Ashraf, et al., 2004; Morcillo and Manzanera, 2021). Soil salinity is mainly caused by NaCl (Gupta and Huang, 2014), so the binding potential of EPS in the alleviation of salinity stress will be explained with the sodium cation in this section. inoculation/co-inoculation of salinity-tolerant For example, exopolysaccharide-producing bacterial species with plant growth promotion on wheat seedlings in saline and non-saline conditions revealed that sodium uptake by wheat plants was significantly reduced by 2.5-6.3% under saline conditions, suggesting that EPS ameliorate salinity stress by limiting available Na<sup>+</sup> uptake into wheat seedlings; consequently, it considerably promotes the root, shoot,

and dry weights of the plants in salty soils (Upadhyay, et al., 2011). Likewise, the study with one of EPS-generating *Pseudomonas* isolates inoculated in soybean plant under saline conditions showed that the EPS-producing strain reduces EC of soil from 1.1 to 0.9 dS m<sup>-1</sup> by chelating free sodium ions in the 200 mM NaCl-treated soil; as a result of sodium absorption, Na<sup>+</sup>/K<sup>+</sup> ratio was lowered in plant tissues and the plant growth was significantly enhanced under salt stress (Kasotia, et al., 2016). In another research, it was found that EPS decrease the concentration of free sodium cations in aqueous solution, emphasizing the importance of EPS in tolerating salinity stress (Nunkaew, et al., 2015).

Another good example was observed in the Na<sup>+</sup> removal potential of EPS produced by a consortium of cyanobacteria in the improvement of wheat, maize, and rice seedlings on salinity tolerance. Arora et al. (2010) demonstrated that application of cyanobacterial EPS markedly decreases the deleterious effect of salinity and improves seed germination in these three crops. Upon addition of the EPS, the chlorophyll content was also improved by up to 56% at 110 mM NaCl treatment (Arora, et al., 2010). In that study, the enhancements in NaCl tolerance upon EPS amendment were attributed to the adsorption capacity of EPS due to the ~50% removal of sodium ions from the aqueous solutions. Similarly, in a more recent study, EPS-producing *Bacillus subtilis* and *Marinobacter lipolyticus* strains increased wheat growth and the plant resistance to salinity stress by absorbing available Na<sup>+</sup> from soil under salinity-stressed conditions (Atouei, et al., 2019). Considering the studies mentioned above, the main role of EPS in the increased tolerance to salinity stress is apparently because of their cation-binding capability.

On the other hand, the study with inoculation of wheat seedlings by EPS-producing *Aeromonas* and *Bacillus* strains under moderate salinity suggested that reduction in Na<sup>+</sup> influx into the plant roots is attributed to the lowered passive influx of sodium cation into the stele rather than to the Na<sup>+</sup> binding potential of EPS because of the higher proportion of root surface covered with soil sheaths in the treated wheat seedlings (Ashraf, et al., 2004).

## 2) Increased soil aggregation around the roots

The second important reason of how EPS ameliorate the inhibitory effects of salinity is through the increased soil aggregation around the plant roots in salt-stressed plants. Former studies have shown that inoculation of roots with rhizobacteria contributes to soil aggregation (Gouzou, et al., 1993; Bezzate, et al., 2000). Treatment of wheat plants with planktonic *Bacillus* 

*polymyxa* resulted in a 57% increase in the mass of root-adhering soil and a more porous structure in rhizospheric soils (Gouzou, et al., 1993). It was believed that the enhanced mass of root-adhering soil with increasing porosity contributes to water retention and nutrient transfer between roots and rhizosphere soils.

The underlying mechanism of microbe-induced soil aggregation is attributed to EPS production. Microbial EPS have been known to increase soil aggregation around the plant roots (Alami, et al., 2000; Vanhaverbeke, et al., 2003; Tewari and Arora, 2014). For instance, inoculation of sunflower by the EPS-producing free-living *Rhizobium* strain increased the mass and macro-pore volume of root-adhering soils by the production of EPS; as a result, it favored the plant growth by relieving the impact of water stress (Alami, et al., 2000). In that study, it is believed that EPS increase water-holding capacity of rhizosphere soil and decrease water loss during osmotic stress as a consequence of the enhanced rhizosphere soil aggregation by the action of microbial EPS.

One of the reasons of osmotic stress is the increased amounts of soluble salts in soils. Salinity stress reduces water and nutrient uptake by plant roots; thereby causing osmotic stress and inhibiting physiological and metabolic attributes of plants (Srivastava, et al., 2019). Salt-induced osmotic stress might be tolerated by the intensified aggregation of root-adhering soil formed by EPS. For instance, application of talc-based EPS formulation significantly enhanced the germination rate and growth parameters of sunflower under salinity stress (Tewari and Arora, 2014). This study suggested that EPS formulations might increase soil porosity and aggregation, water retaining capacity of soils, the nutrient and water uptake by roots, and the quality of soil structure; therefore, stimulating plant growth by mitigating the salt stress. In addition, another study observed that bacterial EPS enhance soil aggregation and chickpea growth under saline conditions (Qurashi and Sabri, 2012a). The potential of EPS in soil aggregation and the amelioration of salt stress were also shown in salt-stressed wheat seedlings by Ashraf et al. (2004). Overall, EPS-producing soil microorganisms contribute to the alleviation of salt-induced osmotic stress as a consequence of the increased mass of root-adhering soils by EPS; consequently, enhancing plant tolerance to salt stress. In the studies above, the capability of microorganisms to form soil aggregates varied from strain to strain (Ashraf, et al., 2004) depending on salt concentrations (Qurashi and Sabri, 2012a); for this reason, it is important to test the performance of the strains in soil aggregation, EPS production, and the efficacy of plant growth promotions

at varying salt concentrations prior to bioformulation applications for the improvements of salinity tolerance.

## 3) Biofilm formation

Biofilms have been defined as microbial communities encapsulated by a self-produced extracellular matrix on a living/non-living surface (Figure 1). Extracellular polymeric matrix-mainly composed of exopolysaccharide, protein, nucleic acid, and lipid-plays a critical role in biofilm formation and confer lots of advantages to the organisms in biofilms (Flemming and Wingender, 2010). Biofilm matrix facilitates the mechanical stability and persistence of biofilms on biotic/abiotic surfaces, forming a cohesive network which temporarily immobilizes biofilm-forming cells by keeping the cells in close proximity for the allowance of cellular and extra-cellular interactions (Flemming and Wingender, 2010). EPS are the main component of biofilm matrix and required for biofilm formation (Danese, et al., 2000; Wingender, et al., 2001; Ma, et al., 2009).

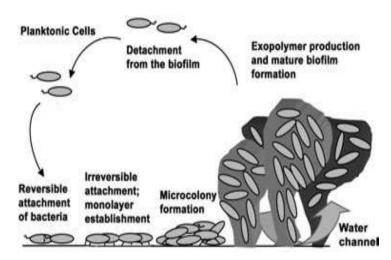


Figure 1 The steps of biofilm formation (Fujishige, et al., 2006).

Biofilm formation depends on numerous factors including salinity (Çam and Brinkmeyer, 2020). Increasing concentrations of salt trigger exopolysaccharide synthesis and biofilm formation in halotolerant *Halomonas variabilis* and *Planococcus rifietoensis* strains, which is attributed to the increased bacterial adhesion on the root surface at higher salinity (Qurashi and Sabri, 2012a) since salt stress increases root adherence and biofilm formation (Ansari et al. 2019). Moreover, previous reports indicated that the elevated levels of salinity increase EPS production, which in turn stimulates biofilm formation. Arora et al. (2010) revealed that salinity induces the production of cyanobacterial EPS. Binding potential of EPS contributes to bacterial adhesion on the surfaces. Attachment and/or adhesion to a surface is the first crucial step during biofilm development (Çam and Brinkmeyer, 2020). Increased cell attachment as a result of EPS production in response to salinity stress triggers biofilm formation on the surfaces. For example, Ala (2018) observed that the enhanced production of EPS in response to increasing salinity promotes biofilm formation by PGPR strains. Similarly, biofilm formation can be favored by the increased EPS synthesis under salt stress (Qurashi and Sabri, 2012a). Supportingly, biofilm formation together with EPS followed a similar production pattern and increased with increasing levels of salinity to some extent (Ansari, et al., 2019).

On the other hand, the amounts of EPS produced under salt stress are not considered an indication of biofilm production since salt influences EPS and biofilm production at different levels (Qurashi and Sabri, 2012b; Morcillo and Manzanera, 2021). The extent of biofilm production might be differently affected by the changing external conditions and the surfaces (Çam and Brinkmeyer, 2020). A study found that the highest biofilm production by some moderately halophilic strains is observed at 1 M NaCl while the maximum amount of EPS is produced at 0–1 M salt concentration (Qurashi and Sabri, 2012b). In that study, biofilm production by the tested strains varied depending on varying salt concentrations, the strains, and the nature of the surfaces. EPS production was also variable according to media composition and substrate concentrations. For instance, the strain-*Halomonas meridiana* PAa6-produced the highest amounts of biofilm at 1 mM salt in plate assays and at 50 mM salt in soil compared to other corresponding strains (Qurashi and Sabri, 2012b).

Inoculation of halotolerant PGPR in improving plant growth by mitigating salinity stress has been well-established (Nadeem, et al., 2013; Vimal, et al., 2017; Kumar, et al., 2021), but such free-living beneficial microbes may be adversely affected by habitat-imposed biotic/abiotic factors (Vimal, et al., 2017; Ansari, et al., 2019). Their plant growth promotion attributes can also be changed in response to varying environmental conditions such as salinity (Upadhyay, et al., 2011; Ansari et al., 2019). Salt also adversely influences the bacterial attachment to plant roots because of the alterations in ionic strength (Jofre, et al., 1998). Adaptation of such microorganisms to stressful conditions is necessary to interact with plants effectively (Choudhary,

2012). It has been estimated that above 99% of bacteria prefer living in biofilm matrix in nature and this lifestyle is also common among rhizobacteria (Wu, et al., 2019). Biofilm formation in response to adverse conditions is also recognized as an important survival strategy adopted by rhizobacteria for their successful root colonization (Kasim, et al., 2016; Ansari, et al., 2019). Biofilm matrix acts as a protective barrier and protects the biofilm-producing cells from various external stresses (Flemming and Wingender, 2010; Cam and Brinkmeyer, 2020). It is also speculated that nutrient competition between microorganisms and salt-induced osmotic stress drives them to live in biofilm mode of growth for protection (Qurashi and Sabri, 2012a). More importantly, hydrophilic EPS provide a hydrated micro-environment that protects biofilm cells from desiccation and increases the survivability of microorganisms under water-stressed conditions by retaining more water around biofilm-forming organisms (Flemming and Wingender, 2010; Kambourova, et al., 2015). Furthermore, biofilm formation in soil considerably increases soil microbial diversity, maintaining a diverse and robust community in soil, protecting soil microorganisms from stress conditions, driving soil microbial metabolic and biogeochemical processes, and also positively influencing physico-chemical properties of soil (Wu, et al., 2019). Application of biofilm-forming PGPR considerably contributes to soil productivity and crop growth under salinity stress conditions (Qurashi and Sabri, 2012a).

Considering the beneficial effects of biofilm formation together with EPS on the viability and diversity of soil microorganisms, soil health and fertility, plant growth stimulation, and other parameters; recent studies have focused on biofilm-forming PGPR strains in improving the tolerance against various stresses including salinity. For example, the inoculation of biofilmforming PGPR enhanced barley growth by alleviating the inhibitory impact of salinity (Kasim, et al., 2016). Likewise, Ansari et al. (2019) observed that biofilm-producing Bacillus pumilis strain with growth promotion stimulates wheat growth parameters by restoring physiological changes and photosynthetic pigments under varying salinity. This strain was able to produce strong biofilms on plant surface as well as microtitre plate and glass surface under NaCl stress. Furthermore, Pseudomonas anguilliseptica SAW24 exhibiting the best biofilm-forming and EPS-producing potential under salinity favored the growth of faba bean under salt stress more significantly than the other treatments (Alaa, 2018). One of our studies also demonstrated that the highest biofilm-forming Azotobacter stains with increasing salinity promote maize growth at 150 mM NaCl stress better than the salt-stressed groups either

uninoculated or inoculated with less biofilm-producing strains (data not published).

## The concentration and composition of EPS

Biofilm matrix is primarily composed of polysaccharides, protein, nucleic acid, and lipid, all of which provide many advantages to biofilm-forming organisms (Flemming and Wingender, 2010). As mentioned previously, several reports indicated that microbial EPS concentration increases with increasing salinity. In addition to its concentration, EPS composition also changes with stress conditions.

Under saline stress, the concentrations of EPS carbohydrates and proteins increase with elevated levels of salinity, which could be used as a selfprotective mechanism for biofilm-producing microorganisms. Tewari and Arora (2014) found a strong relationship between exopolysaccharide synthesis, carbohydrate and protein content, and salt resistance. For instance, up to 1600 mM NaCl, EPS carbohydrate and protein content in EPS-producing P. aeruginosa strain PF07 were progressively enhanced by up to 45.83% and 48.35%, respectively (Tewari and Arora, 2014). They also observed the highest EPS production (by 58.09%) at 1600 mM NaCl compared to the lower levels of NaCl. Besides, another study showed that under stress conditions, microorganisms produce more EPS for protection from osmotic stress (Sandhya, et al., 2009). It was suggested that energy expenditure is devoted to protection from salt stress by enhancing EPS synthesis, which increases the survivability of the cells and mitigates salinity stress (Tewari and Arora, 2014). Additionally, more EPS content and root adhering soil aggregation increase the water and nutrient uptake by plant roots from soils, thus helping plants in alleviating osmotic stress (Sandhya, et al., 2009). Accumulation of proteins under stress might be used for osmotic adaptation during the stress (Singh, et al., 1987). Over-expression of stress proteins may help in membrane stabilization by maintaining osmotic balance of cell membranes and also in the reduction of sodium toxicity by activating osmotic regulatory mechanisms (Tewari and Arora, 2014).

An increment in salt concentration not only increases carbohydrate and protein content but also modifies their compositions. Tewari and Arora (2014) observed that the major EPS subunits were glucose and galactose under below 500 mM NaCl conditions, but were glucose, rhamnose and trehalose above 500 mM NaCl. They also revealed that glucose was the only sugar in EPS at 0 mM NaCl, but under salinity stress, EPS were formed from the different

combinations of galactose, glucose, rhamnose, and trehalose. It was claimed that these sugar components serve as carbon reservoirs, which protects against salt stress and the changes in water potential (Tewari and Arora, 2014). Similarly, in another work, EPS composition was found to be totally different under salt stress compared to non-stress groups. This work demonstrated that EPS composition of *Azospirillum brasilense* was primarily glucose under non-salinity stress; however, under 300 mM NaCl stress, EPS content was largely composed of galactose with about 90% rate, and the proportion of the other sugars was comparatively very low (Fischer, et al., 2003).

It is clear that microorganisms modify their EPS content and composition in response to saline stress. Binding potential of EPS assists soil particles to stick together and with root surfaces, therefore stimulating crop growth and productivity under salinity stress (Morcillo and Manzanera, 2021). Since the sticky nature of EPS is dependent on the varying concentration and composition of different types of carbohydrate, protein, and lipid (Morcillo and Manzanera, 2021), it can be concluded that the potential of EPS in the amelioration of salt stress in plants may underlie its concentration and composition.

## CONCLUSIONS

It is well established that bacterial EPS have a huge potential to ameliorate salinity stress in plants by 1) restricting uptake of soluble salt ions through either cation-binding potential or reduced passive influx of cations, 2) increasing soil aggregation around the roots and soil porosity in rhizosphere, and 3) producing biofilms on root surface or in rhizosphere. Biofilm formation provides many benefits to both rhizobacteria and plants. Biofilm formation together with hydrophilic EPS maintain a hydrated microenvironment which protects biofilm-forming cells from desiccation under osmotic stress by holding more water around the organisms. It also increases soil microbial diversity, maintains a robust community in rhizosphere, protects soil microbes from adverse conditions, drives microbial metabolic attributes, improves physicochemical properties of soil, increases cell adhesion to plant roots, and contributes to plant growth under saline stress. Most of the earlier studies have paid attention to halo tolerant free-living PGPR in the alleviation of saline stress. These planktonic microorganisms and their plant growth-promoting traits may be adversely affected by harsh environmental fluctuations, but biofilm formation protects the biofilm cells from stress conditions. Taken into account the benefits of biofilm formation, it might be better to work with

biofilm-forming PGPR as promising bioinoculants in ameliorating external stresses.

Furthermore, there is a strong correlation between EPS and biofilm production. EPS synthesis drives microorganisms to form biofilms. The salt tolerance mechanisms of biofilms in both microbes and plants mostly rely on the presence of EPS. However, in literature, there is very little knowledge about physico-chemical properties and composition of EPS produced in response to salt-induced stress. Whereas, the concentrations and compositions of EPS largely vary with the different levels of salt. Therefore, studies to increase plant growth and productivity by ameliorating salt stress should mostly focus on the determination of EPS structure and composition together with biofilm-forming ability under not only varying salt concentrations but also other different conditions.

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## **CHAPTER 12**

## DETERMINATION OF LAND AND WATER RESOURCES AND THE PRODUCTION CAPACITY OF SOME FORAGE AND GRAIN FEEDS USED IN ANIMAL NUTRIATION OF TEKIRDAG PROVINCE

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#### **INTRODUCTION**

With the rapid population growth in the world, development of industrial and climate change, importance of land and water resources has gained meaning again. While mentioing about the importance of water in their work in 2007, Konukcu et al. (2007) attracted attention to four main trends as: population growth, urbanization, climate change and economic development and globalization. Besides, water and land resources are two inseparable parts that influence each other. With the increasing population and industrialization, changes have occurred in land use, and fertile agricultural lands were adversely affected with this situation. Soil and water resources are among the most important natural wealth of countries and they comprise the main elements of agriculture that meet the food needs of the country's population. Development, protection and management of soil and water resources has become a necessity today (Kiymaz 2011). The effective use of soil and water resources is also important in terms of sustainability of agriculture and animal husbandry and transferring resources to the future generations.

To be able to solve the nutritional problem of the rapidly increasing world population, animal products are needed rather than plant products, and there is a necessity to feed animals in almost all countries, regardless of their economic situation (Dogan et al. 2003). Livestock has become an industry in developed countries and it has become an integral part of the economy today. This shows that agriculture, and hence animal husbandry, has a strategic importance that needs to be developed at national level (Kirchgessner 1985). But, in most countries, agriculture is the main occupation of a significant portion of the population. It is a well-known fact that livestock has a great weight in agriculture. Another fact that is well-known is related with low animal productivity. An important part of the reasons why the productivity in animal production is far from being at the desired level is mainly due to ignorance and, accordingly, the lack of nutrition (Alcicek et al. 2010, Kirchgessner 1985). Food for animals must provide all necessary nutritional components and energy to maintain the physiological functions being vital for the growth, reproduction and health of animals. Besides, food should be as easy to digest as possible and the negative effects on the environment should be as low as possible (Kirchgessner 1985). Feed expenses constitute nearly 70% of the total costs of an enterprise. 78% of these feed expenses are roughage and 22% are concentrated feed. Quality of the roughage, being abundant and cheap, minimizes the use of concentrated feed, which is

generally more expensive, on the other hand, it provides great economic gain to the relevant business (Harmansah 2018).

With this study, the current data on the land, water resources and some roughage and grain forage used in animal feeding, planting and harvested areas, production amount and yield values of Tekirdag province between the years 2012-2021 were tried to be summarized.

## 1. Location of Tekirdag Province

Tekirdag province is located on the coordinates of 26° 43'- 28° 08' east longitudes, 40° 36'- 41° 31' north latitudes. It's coastline is 133 km to the Sea of Marmara and 2.5 km to the Black Sea, with a total of 135.5 km. Tekirdag, which is one of the 3 provinces of Turkey, all of which are in the European Continent, is located in the northwest of the Sea of Marmara, on lands covered with rich alluvium. It is surrounded by Istanbul in the east, Edirne and Canakkale in the west, the Marmara Sea in the south, Kirklareli in the north and the Black Sea with a short coast. Tekirdag is a modern agricultural and industrial city with fertile lands in the south of Thrace (Anonymous 2022a). Tekirdag has an area of 6.218 square kilometers (Anonymous 2022b). The location of the research area is given in Figure 1.

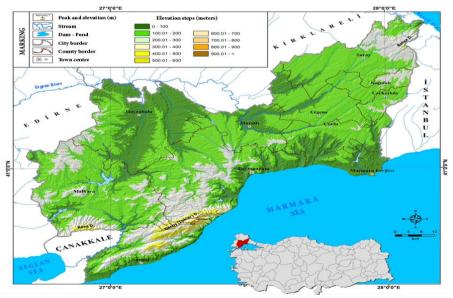


Figure 1. Research area (Ozsahin and Eroglu 2021)

## 2. Climate of Tekirdag Province

The Mediterranean climate is generally dominant on the shores of the Marmara Sea. However, unlike the shores of the Mediterranean Region, snowfall can be seen in the coastal part in winter. In the inner parts of the province, the continental climate is hot in summers and cold in winters (Anonymous 2022a). According to the meteorological data of the research area for many years (1940-2021), presented in Table 1, the annual average temperature is 14.1 °C. In terms of monthly temperature averages, the coldest month is January with 4.8 °C, and the hottest month is August with 23.8 °C. The average highest temperature is 17.9 °C and the average lowest temperature is 10.4 °C. The total annual precipitation is 583.5 mm. The month with the highest average number of rainy days is March.

Months	Average Temperature (°C)	Average Highest Temperature (°C)	Average Lowest Temperature (°C)	Average Radiation Time (hour)	Average Rainy Days Number	Average Monthly Total Rainfall Amount (mm)
January	4.8	8.1	2.0	2.7	10.76	69.5
February	5.5	9.0	2.5	3.3	10.35	54.6
March	7.3	11.1	4.1	4.2	11.65	53.9
April	11.7	15.7	8.1	5.8	8.24	41.1
May	16.7	20.6	12.7	7.3	8.41	37.6
June	21.1	25.3	16.7	8.6	7.82	38.7
July	23.7	28.0	19.0	9.4	3.24	24.2
August	23.8	28.2	19.4	8.5	1.59	15.4
September	20.2	24.5	16.1	6.8	5.35	33.4
October	15.6	19.5	12.1	4.6	9.41	61.4
November	11.3	14.8	8.1	3.2	8.88	73.2
December	7.2	10.4	4.3	2.5	11.12	80.5
Annual	14.1	17.9	10.4	5.6	96.80	583.5

Table 1. Long-term ave	rages of climate valu	les of the research area	(1940 - 2021)

(Anonymous 2022c)

## 4. Land Presence and Usage Status of Tekirdag Province

According to the agriculture report of Tekirdag Provincial Directorate of Agriculture and Forestry, when the provincial land

quality distribution is examined, the total cultivated area for 2021 is 65.80%, forest land is 17.39%, non-agricultural land is 11.51% and pasture land is 5.30% (Anonymous 2022d).

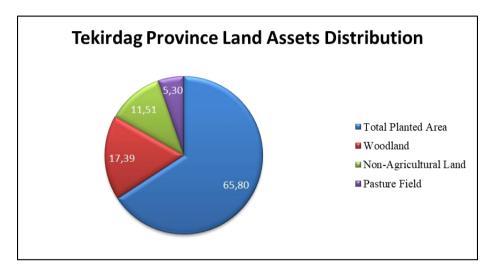


Figure 2. Land quality distribution in Tekirdag province (2021)

According to the data of year 2021 in Tekirdag, the total agricultural area is comprised of 38.063 thousand hectares, the total cultivated agricultural area is comprised of 19.851 thousand hectares, and the cultivated area of cereals and other plant products is comprised of 16.031 thousand hectares. Between the years 2012-2021, the total agricultural area, the total cultivated agricultural area and the cultivated area of cereals and other plant products followed a fluctuating course. On the other hand, the fallow of cereals and other plant products showed a decreasing trend in a 10-year period and reached 3.059 thousand hectares in year 2021 from 4.286 thousand hectares. Between years of 2012-2021, meadow and pasture land showed a constant course and remained at the level of 14.617 thousand hectares. Although forest areas remained stable for some years, it showed an increasing trend from year 2012 to year 2021 and reached 22.933 thousand hectares from 21.678 thousand hectares (Table 2).

Year	Total Agricultur al Area	Total Cultivated Agricultural	Field of Cer Other H Produ	lerbal	Meado w and Pasture	Forest Area
	al Al Ca	Area	Sown Field	Fallow	Land	
2012	38.399	20.581	15.463	4.286	14.617	21.678
2013	38.423	20.574	15.613	4.148	14.617	21.678
2014	38.558	20.699	15.782	4.108	14.617	21.678
2015	38.551	20.650	15.723	4.114	14.617	22.343
2016	38.328	20.382	15.575	3.998	14.617	22.343
2017	37.964	19.998	15.498	3.697	14.617	22.343
2018	37.797	19.723	15.421	3.513	14.617	22.622
2019	37.716	19.580	15.398	3.387	14.617	22.740
2020	37.762	19.586	15.628	3.173	14.617	22.740
2021	38.063	19.851	16.031	3.059	14.617	22.933

 Table 2. Agricultural and forest areas (thousand hectares)

(TUIK 2022)

### 5. Water Resources Availability of Tekirdag Province

The existence of water resources of Tekirdag province has been examined in two parts as surface waters and underground waters.

## 5.1. Surface Waters

Surface water potential of Tekirdag province is 713 hm<sup>3</sup>/year (Anonymous 2022e). The rivers of the province are Ergene River, Hayrabolu Stream, Corlu Stream, Besiktepe Stream, Koca Stream (Cokal), Seymen Stream, Hoşkoy Stream, Kayi Stream and Gazioglu Stream. The river information is given in Table 3. The river with the longest total length is the Ergene River and its length within the borders of Tekirdag province is 85 km.

## 5.1.1. Irrigation, Dams and Ponds of Tekirdag Province

There are Karaidemir, Turkmenli, Ferhadanli, Naipkoy and Çokal dams, Biyikali, Bayramsah, Temrezli, Yazir, Inanli, Ulas, Balabancik, Karacahalil, Hanoglu, Sarkoy, Gazi ponds and Guneskaya regulator in Tekirdag province. These are planned as irrigation, flood, drinking, utility water and animal drinking water. Information about the current irrigation, dams and ponds of Tekirdag province is shown in Table 4.

Stream	Total Length (km)	Length Within Province Boundaries (km)	Flow Rate (m <sup>3</sup> /sn)	Belonging to Stream	Purpose of Usage
Ergene River	220	85	26.49	Meric	Natural Stream
Hayrabolu Stream	55	55	4.37	Ergene Nehri	Karaidemir Dam
Corlu Water	85	85	2.67	Ergene Nehri	Natural Stream
Besiktepe Stream	92.8	92.8	2.04	Ergene Nehri	Natural Stream
Koca Stream (Cokal)	52	44	1.43	Koca Dere	Cokal Dam
Seymen Stream (Karaevli)	16.5	16.5	-	Seymen Dere	Natural Stream
Hoskoy Stream	14.6	14.6	-	Hoskoy Deresi	Natural Stream
Kayi Stream	12.9	12.9	-	Kayi Deresi	Natural Stream
Gazioglu Stream	13.3	13.3	-	Gazioglu Deresi	Natural Stream

(DSI 2021)

## 5.2. Groundwater

There are many wells drilled artificially for industry and industrial irrigation purposes in the settlements. In recent years, uncontrolled drilling of wells has been observed in order to meet the increasing water demand, especially with the development effect of industrialization in the region. However, in order to prevent this situation, The State Hydraulic Works (DSI) Regional Directorate has tried to restrict the drilling of wells. The groundwater level, which was 10-30 m in the 1970s, has decreased to 80-200 meters today. Groundwater throughout the region is used for drinking, use and agricultural irrigation purposes, and in terms of water quality, according to WILCOX evaluation, the total hardness is between 10 and 40 according to the French Hardness degree (Anonymous 2022e).

## 5.2.1. Groundwater Levels

Actual allocation amount of groundwater in year 2020 is 37.1 hm<sup>3</sup>/year as irrigation water, 94.55 hm<sup>3</sup>/year as drinking and utility water, 114.94 hm<sup>3</sup>/year as industrial use, making up a total of 246.59 hm<sup>3</sup>/year. Since the amount of water drawn annually in the region as industrial, drinking and utility water is

higher than the amount of groundwater being produced, it has caused significant decreases in the groundwater level since year 1973. With "Ergene and Meriç Basins Groundwater Operation Announcement" published in the Official Gazette dated 5 November 2009 and numbered 27397, all kinds of groundwater allocations were closed. Groundwater withdrawal is controlled on-line with pre-loaded remote-controlled water meters, and the works are followed by DSI (Anonymous 2022e).

Dam and Pond	Irrigation	Purpose	Irrigation Area (da) Gross	Irrigation Area (da) Net	Drinking Water (hm <sup>3</sup> /year)	Normal Volume (m <sup>3</sup> )	Usable Volume (m <sup>3</sup> )
Karaidemir Dam	Hayrabolu Irrigation	I+F	89.230	77.200	-	109.070.000	108.941.000
-	Ibribey Irrigation	Ι	3.850	3.360	-	Water Source K	araidemir Dam
Turkmenli Dam	Turkmenli Dam Irrigation	I+U	5.710 (measured 4400)	3.450	1.3	14.849.000	14.321.000
Biyikali Pond	Biyikali Pond Irrigation	Ι	3.020	2.550	-	3.589.000	3.236.000
Bayramsah Pond	Bayramsah Pond Irrigation	Ι	1.450	1.230	-	1.911.000	1.629.000
Temrezli Pond	Temrezli Pond Irrigation	Ι	1.250	1.170	-	1.036.000	954.000
Yazir Pond	Yazir Pond Irrigation	I+U	4.000	3.600	1.72	5.450.000	4.784.000
Inanli Pond	Inanli Pond Irrigation	Ι	530	470	-	612.456	525.306
Ulas Pond	-	-	0	0	-	290.000	263.000
Balabancik Pond	-	ADW	0	0	-	100.000	
Karacahalil Pond	Karacahalil Pond Irrigation	Ι	1.900	1.660	-	888.000	800.000
Guneskaya Regulator	Guneskaya Regulator Irrigation	Ι	2.200	1.770	-		
Hanoglu Pond	Hanoglu Pond Irrigation	Ι	7.210	6.490	-	4.630.000	3.850.000
Sarkoy Pond	-	U	-	-	1.053	1.206.000	1.053.000
Ferhadanli Dam	Ferhadanli Dam Irrigation	Ι	4.950	4.455	-	1.682.000	1.658.000
Naipkoy Dam	-	U	0	0	6.43	21.564	19.754.000
Gazi Pond	Gazi Pond Irrigation	Ι	1.550	1.400	-	740.000	672.000
Cokal Dam	-	I+U			7.52	204	
TOTAL			121.140	108.805			
(DSI 2022)	I_ Irrigati	on U- I	Icing Drint	ing Wat	$r T = \overline{\Gamma}$	ood ADW-	- Animal

(DSI 2022) I= Irrigation, U= Using Drinking Water, T= Flood, ADW= Animal Drinking Water

	Irrigation	Irrigated Area (ha)									
Irrigation	Area Net (ha)	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Hayrabolu Irrigation	7.720	4.628	4.618	4.600	5.074	4.111	1.346	4.135	4.188	2.689	2.559
Ibribey Irrigation	336	-	-	148	204	200	150	150	126	118	133
Turkmenli Dam Irrigation	345	283	179	219	207	150	156	142	148	151	162
Biyikali Pond Irrigation	255	138	145	118	172	154	138	120	123	158	97
Bayramsah Pond Irrigation	123	42.5	98	33	32	30	23	15	18	14	11
Temrezli Pond Irrigation	117	41.9	51	50	44	43	35	41	40	35	44
Yazir Pond Irrigation	360	-	-	-	90	97	93	87	99	164	169
Karacahalil Pond Irrigation	166	117	126	102	113	117	143	172	170	166	158
Guneskaya Regulator Irrigation	177	-	-	-	-	-	-	-	-	-	-
Gazi Pond Irrigation	140	-	-	-	-	-	-	140	140	140	140
Hanoglu Pond Irrigation	649	-	-	-	152	130	125	148	135	145	136
Ferhadanli Dam Irrigation	446	-	-	-	-	-	-	-	242	254	260
(DSI 2022)											

Table 5. Irrigated area	s in Tekirdag (hectares)
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(DSI 2022)

## 6. Irrigated Areas of Tekirdag Province

The presence of irrigated areas in Tekirdag province is shown in Table 5. When Table 5 is examined, it is seen that the net irrigation area has been reached in 2020 in Karacahalil Pond irrigation and in 2018, 2019, 2020, 2022 in Gazi Pond Irrigation. In addition, it is seen that the net irrigation area was exceeded in 2018 and 2019 in Karacahalil Pond irrigation, and the net irrigation area could not be reached in other irrigations.

## 7. Status of Some Roughage Produced in Tekirdag Province (2012-2021)

Roughage; it is a material that has a water content of more than 14% in its natural state or a crude cellulose content of more than 16-18% in dry matter. The most important and cheapest feed source for livestock is roughage, low-energy feeds grown in natural conditions that form the main part of the rations of ruminant animals. At the same time roughage; It is suitable for the nutritional physiology of ruminant animals and has great importance in terms of providing satiety in animals. Negative factors such as population growth, climate change, drought, decrease in water resources, development of arable land, urbanization in recent years have caused a decrease in roughage production and an increase in the unit price of products (Ozkan and Demirbag 2016). The way to increase animal production in our country depends primarily on providing the required quality roughage. Even if the desired point cannot be reached, there is a significant improvement in our existing animal breeds. With a good feeding program, it is possible to obtain higher yields even from our low-yielding animals. The main principle of feeding is to ensure that animals receive the nutrients they need with various feeds so that they can continue their normal lives and give the expected yield fully (Ogun ve Polat 1987). Meadows and pastures are very important sources of roughage in animal nutrition. At the same time, these are areas that are absolutely necessary to protect and develop the diversity of flora and fauna and our genetic resources for future generations, and for the effective maintenance of agricultural activities and animal husbandry. Another source of quality roughage is forage crops in field agriculture (Ozkan 2015).

The cultivation areas, harvested areas and production amounts of some forage crops used in animal feeding in Tekirdag are given in Table 6, Table 7 and Table 8. There was no significant difference between the cultivation areas and harvested areas of sainfoin, clover, sorghum, alfalfa, oat and triticale produced in Tekirdag.

The general characteristics of sainfoin, sorghum, alfalfa, oat and triticale, which are some forage crops produced in Tekirdag for use in animal feeding, are briefly summarized.

**Sainfoin:** Sainfoin is used as an important forage plant in the evaluation of barren and dry soils where many cultivated plants cannot grow. It is an indispensable plant in alternation in many regions where water is scarce. In such environmental conditions, there is no other leguminous forage plant that can be grown in place of the sainfoin It is a good soil improvement

plant because the roots go deep, it can grow even in poor soils, and it frees the unfree phosphorus in the soil. It is also used very effectively in erosion control. Since the sainfoin gives plenty of honey extract, it is also a good bee pasture (Anonymous 2010). When we examine the cultivation area and production situation over the years; It is seen that the cultivation area, which was 387 decares in 2012, decreased to 85 decares in 2021 (Table 6). Parallel to this decrease, it is seen that the amount of production decreased from 713 tons to 188 tons (Table 8).

Table 6. Cultivation areas of some forage crops produced in Tekirdag (decares)

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Sainfoin	387	290	290	240	151	153	170	59	110	85
Sorghum	138	65	100	60	25	24	53	178	450	250
Alfalfa	16.928	16.164	18.133	17.650	19.430	21.104	20.559	22.101	20.614	19.324
Oat	4.345	3.675	5.806	5.850	5.790	5.677	7.284	11.564	13.415	19.650
Triticale	253	170	170	170	1.200	983	1.260	2.440	835	640
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(TUIK 2022)

Table 7. Harvested areas of some forage crops produced in Tekirdag (decares)

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Sainfoin	384	290	290	240	151	153	170	59	110	85
Sorghum	138	65	100	60	25	24	53	178	450	250
Alfalfa	16.771	16.164	18.133	17.650	19.430	21.104	20.559	22.101	20.614	19.324
Oat	4.304	3.675	5.806	5.850	5.790	5.677	7.284	11.564	13.415	19.650
Triticale	251	170	170	170	1.200	983	1.260	2.440	835	640

(TUIK 2022)

Table 8. Production amount of some forage crops produced in Tekirdag (tons)

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Sainfoin	713	580	638	528	302	306	345	136	238	188
Sorghum	429	200	340	240	100	96	187	605	1.563	720
Alfalfa	68.093	78.437	79.987	80.787	89.558	97.748	95.707	104.184	97.535	91.472
Oat	4.519	4.223	6.167	9.600	11.925	11.691	16.490	26.206	31.313	50.025
Triticale	406	308	338	293	2.180	1.764	2.646	4.985	1.933	1.540
(THE 202)	2)									

(TUIK 2022)

**Sorghum:** Sorghum sudan grass, which has an important place in forage crop cultivation, which is the most ideal solution for the need for roughage, which is an indispensable input of animal breeding, is one of the plants from which the most green grass per unit area is obtained. It can be fed

to animals as silage as well as green and dry grass. It can also be used as pasture (Ogun and Polat 1987). Its adaptation to difficult terrain conditions is strong, and its development period is summer and early. It is a forage plant that is rich in nutrients, highly productive and contains important components for milk yield. It is drought and heat resistant. Feed efficiency increases with irrigation (Anonymous 2022f). When we examine the cultivation area and production amounts by years in Table 6 and Table 8; The cultivation areas and production amounts have changed over the years, the maximum cultivation area was 450 decares and the production amount was 1.563 tons in 2020.

Alfalfa: As being one of the most important forage crops, alfalfa has a very high feed value. Due to this reason, alfalfa is among the most widely grown forage crops in the world. Alfalfa is generally used as dry grass in animal feed. In recent years, alfalfa silage production has become widespread. Besides, the pellet and flour form of alfalfa are also used. In addition to these, clover is also used as a cover crop and green manure against erosion. Alfalfa is rich in protein, calcium and other minerals, as well as vitamins B, C, D, E and K. Alfalfa is a flowering legume forage plant that can be used as a perennial, green and dry herb. Grass yield is high and although it varies according to the regions, 3 to 8 mowings can be made in a year. Alfalfa affects the physical and chemical properties of the soil in which it is grown in a positive way. Alfalfa is a delicious, high-energy and protein food source for dairy cattle. With the bacteria it contains in its roots, it not only ensures the production of high protein feed in soils where nitrogen is insufficient, but also increases the nitrogen level of the soil. Having a very strong root structure increases its resistance mainly during drought periods (Anonymous 2022g). As it can be seen from Table 6 and Table 8, the maximum cultivation area was 22.101 decares and the production amount was 104.184 tons in year 2019.

**Oat:** It has a high oil content and is rich in oleic and linoleic acids. It contains vitamins B1, B2 and B6 and vitamins A, K and E. It also contains important minerals, micronutrients, antioxidants and sterols. The fatty acid content of oats is beneficial compared to those found in other grains. Compared to barley or corn, oats have 1-3% more crude protein. It is grown for green fodder, silage, hay, grain and whole plant use. It is often used mixed with legumes for feed production. Straw is a basic litter as well as a good roughage for livestock. (Anonymous 2022h). When we examine the change

over the years, the maximum cultivation area was 19.650 decares and the production amount was 50.025 tons in 2021 (Table 6 and Table 8).

**Triticale:** As being a hybrid of wheat and rye, triticale is an amphidipioid grain. Triticale cultivation gains importance in terms of reducing the use of wheat, which is used in large quantities in the feed industry, in animal rations, and saving on expensive products such as corn, soybean and similar ones. Being more durable than wheat and barley in all kinds of conditions and giving more products from them makes triticale become an alternative product. When Triticale is used as silage or in the form of concentrated mixtures, it increases the energy level and protein ratio of the ration. This rate is higher than other grains except for oats. Studies on the digestibility of the stems show that triticale is equivalent to wheat and barley (Fernandez et al. 2000). When the change over the years in Table 6 and Table 8 is examined, it is seen that maximum cultivation area was 2.440 decares and the production amount was 4.985 tons in year 2019.

Yield values of sainfoin, sorghum, alfalfa, oat and triticale between 2012-2021 are shown in Table 9. According to Table 9, in the last period of ten years, the yield of sainfoin varied in the range of 1.857-2.305 kg/da, the yield of sorghum varied in the range of 2.880-4000 kg/da, the yield of alfalfa varied in the range of 4.060-4.853 kg/da, the yield of oat varied in the range of 1.050-2.546 kg/da, and the yield of triticale varied in the range of 1.618-2.406 kg/da.

When Figure 3 is examined, it is seen that highest yield in sainfoin was obtained in year 2019, while the lowest yield was obtained in year 2012. While the same and highest yield was obtained in sorghum in years 2015, 2016 and 2017, the lowest yield was obtained in year 2021. In 2013, the highest yield (4.853 kg/da) was obtained in alfalfa, and a slightly increasing trend was observed in the yield in the following years starting from 2013 and the yield value in 2013 was never reached. In a similar way, in oats and triticale, the lowest yield values occurred in 2012, while the highest yield values occurred in 2021, and yield values of both plants followed a fluctuating course over the years.

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Sainfoin	1.857	2.000	2.200	2.200	2.000	2.000	2.029	2.305	2.164	2.212
Sorghum	3.109	3.077	3.400	4.000	4.000	4.000	3.528	3.399	3.473	2.880
Alfalfa	4.060	4.853	4.411	4.577	4.609	4.632	4.655	4.714	4.731	4.734
Oat	1.050	1.149	1.062	1.641	2.060	2.059	2.264	2.266	2.334	2.546
Triticale	1.618	1.812	1.988	1.724	1.817	1.795	2.100	2.043	2.315	2.406
(TUIK 2022)										

Table 9. Yield value of some forage crops produced in Tekirdag (kg/da)

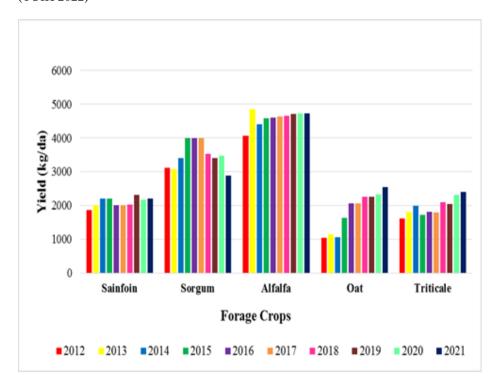


Figure 3. Yield value of some forage crops produced in Tekirdag (kg/da)

Ovine and bovine animals belonging to Tekirdag province are given in Table 10. Between years of 2012-2021, number of sheep and goats showed a fluctuating variability. The quality roughage requirement of animals raised in Tekirdag province in 2021 can be calculated as follows: For a 500 kg cattle, the survival rate crude protein (CP) requirement is 370 g and the metabolic energy (ME) requirement is 14.000 kcal (NRC 1989). Accordingly, nearly 4 kg/day of quality hay and 10 kg/day of quality silage (corn) are required to meet the nutrient requirement of 1 cattle unit (BBHB). Considering the yearround supply of survival share needs, a total of 2.624.337 quality roughage requirements will be met in 2021 as 4 kg x 519.043 BBHB x 365 days = 757.802 tons of hay and 10 kg x 519.043 BBHB x 365 days = 1.894.506 tons of silage/green, which comes out to be a ton.

	Bovine Animal	Ovine Animal
2012	158.596	263.185
2013	154.737	237.540
2014	154.663	309.237
2015	139.053	288.028
2016	140.688	302.399
2017	154.207	310.876
2018	150.056	302.106
2019	149.238	309.073
2020	151.011	321.376
2021	154.072	364.971

Table 10. Number of bovine and ovine animals (head) in Tekirdag

(TUIK 2022)

# 8. Status of Some Grain Feeds Produced in Tekirdag Province (2012-2021)

Seeds and fruits, which form the reproductive organs of cultivated plants, are rich in digestible nutrients and the nutrients they contain are highly digestible. Due to these properties, they are used as human food as well as animal feed. 60-80% of the rations prepared for animals consist of grain feeds or their by-products (Ergun et al. 2002).

The cultivation areas, harvested areas and production amounts of some grain forage crops used in animal nutrition produced in Tekirdag are given in Table 11, Table 12 and Table 13. There was no significant difference between the cultivation areas and harvested areas of oats, barley, wheat, rye, triticale and corn produced in Tekirdag. The general characteristics of oat, barley, wheat, rye, triticale and corn, which are some grain forage crops produced in Tekirdag for use in animal feeding, are briefly summarized.

**Oat:** It is a cereal grain consumed as human food and animal feed in the world. It is a spelt feed substance that is usually given to animals by breaking or crushing. Although it is a suitable feed for dairy cows, it is not

used alone. It has a positive effect on milk yield and milk fat. It is not recommended for use in sheep and goats due to its husk content. (Ergun et al. 2002). Tablo 11 ve Tablo 13'ten de görüleceği üzere en fazla ekim alanı Therefore, it is not recommended for use in sheep and goats. In 2021, 7.828 decares of cultivation areas and production amount were obtained as 3.472 tons.

**Barley:** In addition to being fed to all animals, it is a very tasty feed especially for ruminants and is a mostly used feed ingredient. Up to 50% can be added to the mixed feed of barley dairy cattle. The amount of use in beef cattle can be increased up to 70%. It is a very valuable grain feed for beef cattle in the finishing period. Due to the high corn prices in our country, this grain constitutes a large part of the beef cattle rations (Ergun et al. 2002). It provides very good live weight gain in these animals. When Table 11 and Table 13 are examined on a yearly basis, ups and downs were observed in terms of cultivation areas and production. While the highest cultivation area was 172.380 decares in 2015, the highest production amount was 86.057 tons in 2021.

Wheat: As one of the most basic products of our country's agriculture, it is very important in terms of nutrition of people as well as being fed to animals and converted into animal products. It is very rich in carbohydrates. It is used as an energy source in ruminants. It is highly digestible in ruminants. The rapid digestion rate of starch and the fact that the protein is mainly composed of gluten make it difficult to use wheat in feed compared to other grains. It can be used in dairy cow mixed feeds at the rates of 30-50%, in lamb rations at 25%, in sheep rations at 35%. In Tekirdag province, the most produced grain feed showed a fluctuating course in terms of cultivation areas and production amount on years (Table 11, Table 13). While the maximum cultivation area was 1.966.333 decares in year 2020, highest production amount was 1.026.611 tons in year 2021.

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	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Oat	3.700	3.487	2.931	5.320	6.950	5.494	4.164	6.087	6.503	7.828
Barley	154.120	143.840	146.597	172.380	155.440	130.549	133.288	133.536	134.948	152.800
Wheat	1.593.275	1.683.887	1.724.123	1.841.841	1.924.983	1.922.560	1.877.991	1.899.117	1.966.333	1.924.125
Rye	1.250	1.433	1.119	1.170	1.585	1.375	1.592	802	655	600
Triticale	2.800	2.056	2.965	3.372	2.517	2.959	3.337	4.004	4.092	4.527
Corn	1.775	1.136	1.225	1.278	1.294	581	556	774	770	1.225
	0000									

Table 11. Cultivation areas of some grain forage crops produced in Tekirdag (decares)

(TUIK 2022)

Table 12. Harvested areas of some grain forage crops produced in Tekirdag (decares)

	0010	2012	0014	2015	2016	2015	0010	2010	2020	2021
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Oat	3.700	3.487	2.931	5.320	6.950	5.494	4.164	6.087	6.503	7.828
Barley	154.120	143.840	146.597	172.210	155.440	130.549	133.288	133.536	134.948	152.800
Wheat	1.593.275	1.669.649	1.714.123	1.829.499	1.924.983	1.922.560	1.877.982	1.899.117	1.966.333	1.924.125
Rye	1.250	1.433	1.119	1.170	1.585	1.375	1.592	802	655	600
Triticale	2.800	2.056	2.965	3.372	2.517	2.959	3.337	4.004	4.092	4.527
Corn	1.775	1.136	1.225	1.278	1.294	581	556	774	770	1.225
(TUIK	2022)									

**Table 13.** Production amount of some grain forage crops produced in Tekirdag (tons)

2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
1.380	1.306	1.137	1.865	2.444	1.908	1.318	2.201	2.456	3.472
73.593	63.422	69.908	75.929	69.026	71.575	45.135	65.025	54.013	86.057
779.347	631.164	737.284	744.257	825.714	882.674	637.685	857.020	769.915	1.026.611
550	645	623	560	796	833	657	369	288	302
1.296	921	1.282	1.401	1.017	1.306	1.023	1.462	1.696	2.259
897	646	725	707	826	359	327	451	473	782
	1.380         73.593         779.347         550         1.296	1.380         1.306           73.593         63.422           779.347         631.164           550         645           1.296         921	1.380         1.306         1.137           73.593         63.422         69.908           779.347         631.164         737.284           550         645         623           1.296         921         1.282	1.380         1.306         1.137         1.865           73.593         63.422         69.908         75.929           779.347         631.164         737.284         744.257           550         645         623         560           1.296         921         1.282         1.401	1.380       1.306       1.137       1.865       2.444         73.593       63.422       69.908       75.929       69.026         779.347       631.164       737.284       744.257       825.714         550       645       623       560       796         1.296       921       1.282       1.401       1.017	1.380         1.306         1.137         1.865         2.444         1.908           73.593         63.422         69.908         75.929         69.026         71.575           779.347         631.164         737.284         744.257         825.714         882.674           550         645         623         560         796         833           1.296         921         1.282         1.401         1.017         1.306	1.380         1.306         1.137         1.865         2.444         1.908         1.318           73.593         63.422         69.908         75.929         69.026         71.575         45.135           779.347         631.164         737.284         744.257         825.714         882.674         637.685           550         645         623         560         796         833         657           1.296         921         1.282         1.401         1.017         1.306         1.023	1.380         1.306         1.137         1.865         2.444         1.908         1.318         2.201           73.593         63.422         69.908         75.929         69.026         71.575         45.135         65.025           779.347         631.164         737.284         744.257         825.714         882.674         637.685         857.020           550         645         623         560         796         833         657         369           1.296         921         1.282         1.401         1.017         1.306         1.023         1.462	1.380         1.306         1.137         1.865         2.444         1.908         1.318         2.201         2.456           73.593         63.422         69.908         75.929         69.026         71.575         45.135         65.025         54.013           779.347         631.164         737.284         744.257         825.714         882.674         637.685         857.020         769.915           550         645         623         560         796         833         657         369         288           1.296         921         1.282         1.401         1.017         1.306         1.023         1.462         1.696

(TUIK 2022)

**Rye:** It is easily digested by animals by means of the nutrients it contains. It is a feed that is rich in energy content. No more than 20% is used in fattening, especially in dense feeds. It can be added up to 40% of mixed feed in cattle. Sheep consume rye more than other animals. It is suggested to be used with barley, oats and corn in order not to cause digestive disorders (Anonymous 2022i). As it can be seen in Table 11, cultivation areas followed a fluctuating course until year 2018, and a significant decrease was observed

in years of 2019, 2020 and 2021. While the highest cultivation area was 1.592 decares in year 2018, the highest production amount was 833 tons in year 2017 (Table 11 and Table 13).

**Triticale:** Triticale is a grain feed obtained by crossing wheat and rye. In this crossing, it is aimed to combine the grain quality, productivity and disease resistance of wheat with the grain hardness and high lysine content of rye. The feed value of triticale as an energy source is comparable to maize and other grain feeds. Its digestibility is similar to that of wheat. It is an alternative to corn and wheat. Dairy cows and beef cattle can be used up to 30%. Triticale is richer than wheat and rye in terms of the amount of phosphorus in the grain (Ergün et al. 2002). When we look at the cultivation area and production amounts by years, maximum cultivation area was 4.527 decares and the production amounts showed variation (Table 11, Table 13).

**Corn:** It contains high level of starch (%65). It ferments more slowly than other cereal grain starches. It is a suitable feed for all animals and also a very valuable energy source. Since its starch is broken down slowly in the rumen, its acidogenic value is low and 30% is bypassed. Thus, it provides glucose absorption in the small intestine. It can be used for dairy cows and beef cattle (Ergun et al. 2002). According to Table 11 and Table 13, cultivation areas and production amounts generally tended to decrease until 2021 compared to 2012, then a significant increase is seen again in 2021 compared to other years. The highest cultivation area was 1.775 decares and the production amount was 897 tons in 2012.

Yield values of oats, barley, wheat, rye, triticale and corn between 2012-2021 are shown in Table 14. In the last decade, the yield values of oats, wheat, rye, triticale and corn were found to be 317-444 kg/da, 339-563 kg/da, 340-534 kg/da, 413-606 kg/da, 307-499 kg/da 505-638 kg/da, respectively.

	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Oat	373	375	388	351	352	347	317	362	378	444
Barley	478	441	477	441	444	548	339	487	400	563
Wheat	489	378	430	407	429	459	340	451	392	534
Rye	440	450	557	479	502	606	413	460	440	503
Triticale	463	448	432	415	404	441	307	365	414	499
Corn	505	569	592	553	638	618	588	583	614	638
(TUIK 2022)										

Table 14. Yield value of some grain forage crops produced in Tekirdag (kg/da)

When Figure 4 is examined, it is seen that the highest yield in oat, barley, wheat and triticale was obtained in 2021 and the lowest yield in 2018. In 2017, the highest yield (606 kg/da) was obtained in rye, while a fluctuating trend was observed in yield in other years. In the corn, the highest yield value was reached in 2016 and 2021, while the lowest yield was obtained in 2012.

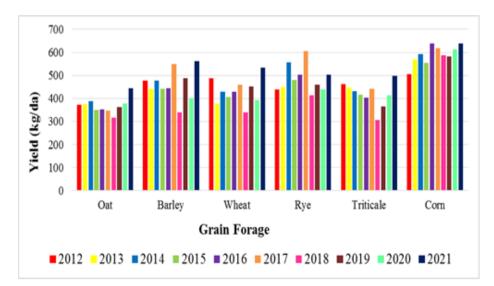


Figure 4. Yield value of some grain forage crops produced in Tekirdag (kg/da)

# CONCLUSION

With this study, firstly, the land existence, usage status, existence of water resources, the existence of irrigated areas and their change over time in Tekirdag province were tried to be revealed. While conducting the study, various data were obtained not only from one institution but also from many

institutions and organizations. Hence, efficient use of land and water resources could be possible with inter-institutional cooperation. Universities, Provincial Directorates of Agriculture, Provincial Directorates of Environment and Urbanization, Municipalities, State Hydraulic Works should be administered with a management style in which the public participates, both at the level of provincial directorates and ministries take part. Furthermore, land and water resources should be managed with "Integrated Basin Management" as constituting two important inseparable elements.

Irrigation applied in agricultural production is the delivery of the part of the water needed by the plant that cannot be met by precipitation to the root of the plant in the required amount and time. In Tekirdag, the Mediterranean climate is generally dominant on the shores of the Marmara Sea, and the continental climate is dominant in the inner parts of the province, and the total amount of precipitation during the year is 583.5 mm. In the province of Tekirdag, there is a total agricultural area of 38.063 thousand hectares in year 2021, while the total net irrigation areas are 10.834 hectares, only 3.869 hectares of land can be irrigated in year 2021. Considering that today's water resources are decreasing, it is necessary to plan irrigation systems and adopt them to producers by using water in the most economical way with the most effective irrigation methods being developed.

Tekirdag province is very suitable for agriculture in terms of climatic conditions and soil structure. Yield amounts obtained in plant production are generally above the Turkey average levels. Besides, animal husbandry is extremely important in Tekirdag province. One of the most important problems in Tekirdag is related with the use of fertile agricultural lands for non-agricultural purposes. The development of industry from Istanbul to Tekirdag, the increase in factories, the increasing population and the irreversibly irregular and distorted construction on fertile agricultural lands has led to a decrease in I. and II. class agricultural lands and water resources. Especially the Ergene river and other rivers, which are 85 km long and have the highest flow rate within the borders of our province, are polluted with petrochemical wastes, industrial and domestic wastes, and also cause pollution of the environment. In addition to these problems, there is also the existence of lands that are fragmented, scattered, and parcels with damaged parcels in such a way that agricultural activities cannot be sustained. The scattered and fragmented agricultural lands in Tekirdag should be integrated with land consolidation. In addition, the integrated lands should be made more productive by supporting investments such as soil improvement and irrigation.

Considering that both climate change and pollution of land and water resources cannot be controlled and water resources are limited, it is very important to determine the temporal variation of land use in order to plan and use resources accurately and effectively. Sustainable use of soil and water resources is important for future generations.

At the same time, with this study, the status of some roughage and grain feeds produced in Tekirdag province and their change over time were tried to be revealed. Forage crops increase the amount of organic matter in the soil, improve the water holding capacity and permeability of the soil, increase soil fertility and suppress harmful and weeds. In this way, the use of chemicals in the soil is reduced and environmentally friendly and sustainable agriculture is provided. Grain feeds used in rations are as important as roughage. It is also used in human nutrition. 60% of grain feed production is provided with imported inputs. Hence, it is costly. Feed expenses constitute 60-70% of the cost in animal feeding. In order for livestock to be profitable, it is necessary to give importance to the production of quality roughage. The low cost of roughage such as hay, green fodder and silage fodder constitute an important factor that increases the profitability of livestock enterprises. Therefore, in order to meet the quality roughage needs of livestock enterprises, it is necessary to improve meadows and pastures, to increase forage plant production areas, and to bring cheap and alternative roughage sources to animal production. Furthermore, agricultural extension tools should be used to transfer quality roughage production techniques to producers, and cooperation should be made with relevant institutions, organizations and universities. In addition, incentives for the use of certified seeds in forage crop production should be increased.

Considering the change in the land and water resources of Tekirdag province and the production status of some roughage and grain forage crops used in animal feeding between the years 2012-2021, it is important and necessary especially in terms of making future plans and protecting natural resources. For the sustainable management of land and water resources, not only as a province of Tekirdag, but also on a regional or even country basis, it is necessary to protect natural resources, try to prevent all kinds of pollution, monitor, control and control and minimize pollution. Effective and efficient use of land and water resources is very important for the sustainability of plant production and animal husbandry, in terms of transferring it to future generations.

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# **CHAPTER 13**

# THE POTENTIAL EFFECTS OF FOODS ON THE REDUCTION OF COVID-19 SYMPTOMS

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# **INTRODUCTION**

### COVID-19, Its Ways Of Transmission, And Human Health

Around 9000 BC, humans living in caves and making their living by hunting and gathering left the caves, migrated to the watersides, established a settled order there, and started agriculture and animal husbandry. This made the transmission of infectious diseases from animals to humans more possible. As humanity developed and time passed, new villages and towns were established. This situation led to the easy spread of epidemics among the people living in these places, and societal deaths were observed. Situations such as migrations, trade, and wars have caused the spread of diseases to wider masses and between societies.

Epidemics have been seen since the beginning of human history and have affected humanity in many ways. There have been many losses and many changes. Historians have recorded numerous outbreaks (Ahmadi et al., 2020).

Some epidemics, which have a great impact on humanity and are important turning points in history, are plague epidemics, Russian flu, Spanish flu, and Asian flu epidemics (Ahmadi et al., 2020). Some of them are explained below.

*Plague of Athens* (430 BC): The first plague epidemic emerged during the Peloponnesian War in 430 BC and spread first to Libya, Ethiopia, Egypt, and later to the Spartans and Athenians, killing 75,000 to 100,000 people (Bryne, 2008; Littman, 2009).

*The Plague of Justinian (541-542 AD)* originated in Egypt, spread to Byzantium and Palestine, and later affected all Mediterranean port cities. The Byzantine emperor Justinian (527-565 AD), who caught this plague and did not die, is the father of this epidemic (Tavukcu et al., 2020). According to some historians, this plague is one of the deadliest epidemics because it killed between 25 and 100 million people. This is considered the first recorded outbreak of the plague. It is a disease caused by the bacterium Yersinia pestis (Bryne, 2008).

*Black Death*: It first reached Asia in the 1330s, when bacteria, fleas, and animals made their way to the Mongolian settlement. About 5 million people died in China (1331), and Mongol armies reached the Genoese city of Kefe with trade (1346). When the Tatars threw the plague-infested corpses into Kefe during the war, the disease became effective in the city. The Genoese who fled the city carried the disease to Europe (Akın, 2012). It caused the death of

200 million people in total in the 14th century (BBC, 2021). This disease was the plague caused by Yersinia pestis first seen in history (Smith, 2020).

*Russian Flu (1889-1890):* This disease, which first appeared in Russia, spread to Asia. From here, it spread to African and American countries and all of Europe (Hays, 1938). Approximately one million people died in this epidemic worldwide (University of Glasgow, 1893).

*Spanish Flu (1918- 1920):* It appeared in 1918 in Kansas, USA. The H1N1 virus is the causative virus. Approximately 500 million people became infected with this virus (Hays, 1938; Poultry et al., 2020). The First World War accelerated the spread of the disease. The Spanish flu killed 50 million people worldwide (Hays, 1938).

*Asian Flu (1957-1958):* This disease first appeared in Hong Kong and then spread to China, Singapore, the USA, and England (Tavukcu et al., 2020). The causative virus of the disease is Influenza A virus H2N2 subtype (Black, 2020; William R., 2008). The epidemic caused the death of approximately 1-2 million people in the world (Jackson, 2009; Black, 2020).

The most important epidemic that has occurred in the recent past is the COVID-19 pandemic, which has greatly affected our life. Before providing information about the coronavirus pandemic, it would be useful to define coronaviruses.

Coronaviruses are included in the subfamily Coronavirinae, belonging to the family Coronaviridae and the order Nidovirales. The structure of these viruses is single-stranded and positively polarized, and their RNA is enveloped. These viruses are called corona due to their structure, which means crown in Latin (Alp and Ünal, 2020). They are classified into four subgroups, alpha-, beta-, gamma-, and delta-type coronaviruses. This classification, which has been previously made with a serology-based approach, is now made with phylogenetic analysis (Chen et al., 2020).

The contamination of coronaviruses starts by holding the virus on hosts in animals, and when suitable conditions are provided, they are transmitted from animals to humans. When viruses are transmitted, they cause gastrointestinal and respiratory infections. Some types of coronavirus show milder symptoms in humans, and the disease is generally easier to overcome. HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 are the most important types of coronavirus. However, these may rarely cause severe symptoms. Severe cases of coronavirus are, for example, the SARS epidemic in 2003 and the MERS epidemic in 2012 (Rothan and Byrareddy, 2020; WHO, 2020). In 2019, an outbreak of pneumonia caused by COVID-19 occurred in the city of Wuhan in China's Hubei province. The cause of this outbreak could not be understood despite the clinical tests. To collect more information, studies were carried out on people showing these symptoms. As a result, the World Health Organization declared that the virus causing these cases was a novel coronavirus on 07.01.2020 (Alp and Ünal, 2020; ICTV, 2020; WHO, 2020).

Although the source of transmission of the epidemic caused by the new type of coronavirus is not yet known exactly, the research has demonstrated that it settles in animals with the potential to be hosts, such as anteaters and bats, and mutates there (Zhang et al., 2020). The mutated virus is transmitted to humans through these animals. Animal markets in China have been shown as the first place of transmission of the epidemic (Lv et al., 2020).

Although the disease is of animal origin, it has been determined to be transmitted from person to person, and over time, the way of transmission from person to person has become the main source of transmission. As the transmission continues and increases, the epidemic has become a global pandemic. It is concluded that coronavirus has many ways of transmission. The ways of transmission of coronavirus are similar to those of influenza viruses because these two species are transmitted by droplets. Mucous saliva and droplets released by an infected person by coughing, sneezing, or talking can infect other people. The virus can also be transmitted by touching the mouth, nose, and eyes of the people who have come into contact with the droplets shed by patients (Türken and Köse, 2020). Coronavirus can also be found in people without any symptoms (WHO, 2019).

Coronavirus is also found in blood count and urine/serum samples (Kurtulus and Özlu, 2020).

The rate of reproduction of the virus is variable. In this case, it is necessary to calculate the reproduction rate of the virus. Another important thing is how long the virus incubation period is, how long it is contagious and how it is transmitted. Moreover, criteria such as the age of people infected by the virus, the population of the region where the virus is seen, and the geographical characteristics of the region are also important for determining the rate of proliferation of the virus (Akın, Gözel, 2020). The duration of coronaviruses in the external environment depends mainly on the type of surface contaminated by the virus. A study showed that the virus could remain alive on metal, glass, and plastic surfaces for up to 9 days (Kampf et al., 2020). Another study found that the virus lived on cardboard for up to 24 hours. Moreover, the virus remained alive and active in snow for up to 4 hours on

copper surfaces and up to 3 days on plastic and stainless steel surfaces (Doramalen et al., 2020).

Coronavirus may show symptoms within 2-14 days after the person is infected. The common symptoms of the disease are high fever, shortness of breath, and dry cough. Additionally, coronavirus also shows symptoms, such as body joint pain, fatigue, headache, sore throat, weakness, abdominal pain, and diarrhea. Furthermore, loss of taste and smell also occurs (Weng et al., 2021).

A study investigated the incidence of the symptoms of coronavirus and determined the following rates of the symptoms: 83-98% for fever, 76-82% for cough, and 11-44% for muscle pain. Weakness and fatigue were also observed among symptoms (Del Rio and Malani, 2020).

When coronavirus is transmitted to people, it primarily invades the respiratory system and causes severe respiratory distress in severe cases, causing pneumonic symptoms such as fever, cough, and sore throat. However, it can also infect other systems of people. It can infect the nervous system and cause dizziness, confusion, and headache. When coronavirus affects the digestive system, diarrhea, abdominal pain, and loss of appetite are observed. When it affects the cardiovascular system, heart injury and chest pain may occur.

COVID-19 is determined using the real-time polymerase chain reaction (PCR). The samples taken from the mucous membranes of the nose and mouth are examined in laboratories, and the presence or absence of the virus is determined. People whose tests show positive results are called COVID-positive. RT-PCR is based on determining the presence of nucleic acid belonging to the SARS-COV-2 virus in the patient's sample (WHO, 2019). This test aims to detect and reproduce different regions in the genome of the virus with known nucleotide sequences. To this end, specially designed primer sequences, Taq polymerase enzyme, appropriate buffer solutions, and dyes with the ability to bind to the copied genetic material are used. On the other hand, tests conducted with urine, blood, and stool samples show low sensitivity and reliability. Therefore, they cannot be used as samples to determine the presence of COVID-19 in the human body (Temel et al.).

Considering the distribution of the number of cases in the world at certain intervals during the pandemic, the number of COVID-19 cases in the world reached 2.5 million on April 20, 2020. COVID-19 was declared a pandemic within 1.5 months from the beginning of the epidemic. After June 3, 2020, the number of cases detected globally reached 6.5 million (WHO, 2020).

While the total number of deaths was 170 thousand until April 20, 2020, it increased to 383,000 from April 20 to June 3. During this period, the number of cases increased by 160%, and the death rate by 125% (Yılmaz, 2021).

Nowadays, approximately 15 million people have been infected with the virus, and approximately 6 million have died. When the clinical findings of COVID-19 are examined, it is observed that the disease is more severe and fatal in the elderly over 65 years of age and people with chronic diseases. On the other hand, it is milder and less common in children. However, the average age of people added to the number of cases has shifted to younger ages as the pandemic progresses. The virus can cause a severe disease process and even death in young people without a chronic disease. In severe and fatal cases, dyspnea (shortness of breath) begins at the end of the first five days, and after this period, the level of oxygen in the blood decreases. As a result, a lack of oxygen in the blood arrives at the molecular level. By the 10th day, the patient is taken to intensive care. Deaths are seen between the 21st and 28th days. In severe cases, 30% of cases develop ARDS (acute respiratory distress syndrome) within eight days, 17% require invasive mechanical ventilation, and 4% require extracorporeal membrane oxygenation (ECMO) (Karadağ et al., 2020). It has been found that antibodies are formed in people who have had COVID-19 and these antibodies persist in the serum and saliva for at least three months. Although rare, when people who have had the disease catch the virus again, they usually exhibit milder clinical symptoms. However, it has also been observed that the second case had a severe course and even resulted in death. Lung problems may occur in some patients who have recovered. Mortality in COVID-19 is 2.34%, which is lower compared to SARS and MERS. The mortality rate rises to 14-15% when only hospitalized cases are taken into account. However, studies have determined an increase in the rates of mortality and morbidity (Karadağ et al., 2020).

In the fight against the disease, it is primarily necessary to follow the rules of vaccination and hygiene, wearing a mask, and social distancing. Hands should be kept clean and washed regularly, surfaces should be cleaned with alcohol, and rules of wearing a mask and social distancing should be followed in crowded areas. However, when the virus is caught, various treatment methods should be applied, and the disease should be overcome mildly (Şenyiğit, 2021).

When treating the disease, severe cases are treated in hospitals, while mild cases are treated with home remedies and natural nutrition. It is known that nutrition has a great impact on immunity and a healthy diet positively affects the immune system, while inadequate and unbalanced nutrition deteriorates the immune system functions and increases the susceptibility to infections. Therefore, in the prophylaxis of COVID-19, ensuring adequate and balanced nutrition of people and meeting their vitamin and mineral needs are essential for the normal functioning of the immune system. This review will examine the effects of nutrition and food groups on the reduction of symptoms during the disease. Due to their contribution to the healing of symptoms or immune-enhancing effects against COVID-19, several food groups such as fruits, vegetables, cereals, meat, fish, dairy products, oils, herbs, probiotics, vitamins, and minerals will be evaluated and discussed.

# 1. IMPORTANT FOOD GROUPS AND COVID-19

# 1.1. Fruits / Vegetables and COVID-19

Among the foods reducing the effect of COVID-19, the role of fruits and vegetables is important. It is known that loss of appetite, inadequate nutrients, and fever adversely affect human health. To reduce the effect of these symptoms, attention should be paid to the healthy nutrition and energy of cases infected with COVID-19 and their consuming foods with high nutritional values, vitamins and minerals.

During the pandemic, the WHO (World Health Organization), Turkish Dietetic Association, and FAO (Food and Agriculture Organization) published nutritional recommendations to deal with COVID-19. They declared that the regular consumption of fruits and vegetables, which should be consumed daily, reduces the effects of the disease and strengthens immunity. Obesity is observed in individuals with a body mass index of 23 and above. These people have a higher risk of contracting COVID-19 and experience the disease more severely than non-obese people. These people should pay attention to their eating and drinking habits and the consumption of fresh fruits and vegetables.

# 1.1.1. Some Important Fruits

Citrus fruits: Citrus fruits contain phytochemicals, vitamins and minerals beneficial to health. Important citrus fruits are oranges, lemons, tangerines, and limes. They contain vitamin C, folic acid, potassium, and pectin, which help to regulate and strengthen the immune system (Felsenstein et al., 2020).

Aonla: This fruit is rich in vitamin C and contains energy for the immune system in humans. It also contains polyphenols and antioxidants. The

consumption of this fruit positively affects the digestive system and health of humans. Moreover, this fruit is rich in phyllemblic acid, gallic acid, ellagic acid, and flavonoids that prolong human life. It also contains vitamin C (500-600mg/100g) and has high antioxidant properties. Hence, De and De (2020) emphasized that the aonla fruit reduces the effect of COVID-19 (De and De, 2020).

Watermelon: A mature watermelon increases the lycopene level in humans and prevents infections that can occur in the liver. Watermelon also contains vitamins, such as B6, C, and A (Baidya, 2020).

Red fruits: Fruits such as blueberries, grapes, strawberries, raspberries, blackberries, and cherries are categorized as red fruits. Red fruits that contain antioxidants are very beneficial for health. Examples of antioxidants include polyphenols in strawberries, ellagic acid in raspberries, and flavonoids in blueberries. Red berries also contain many vitamins and minerals and are rich in potassium, manganese, and fiber.

Papaya: Papaya contains high amounts of minerals, such as iron and calcium, and vitamins, such as vitamins A, B, and C, that support the immune system. Additionally, papaya also contains carpaine, which affects the nervous system. Papaya lowers the heart rate and blood pressure. It is rich in folic acid and ensures amino acid formation from hemocysts in humans. If amino acids are not formed, hemocysts may cause many health problems such as stroke in humans and heart attack due to a high heart rate.

Pomegranate: It is known that pomegranate and pomegranate juice are good foods for cardiovascular diseases. The pomegranate fruit contains vitamins C and E, helping to prevent inflammation. Moreover, it increases the level of antibodies in human blood.

Apple: Apple is a fruit often consumed against obesity. It helps with weight loss and protects the body's energy by increasing the immune system. It also has prebiotic fibers.

# **1.1.2. Some Important Vegetables**

Beetroot: It increases the number of white blood cells in human blood due to the high iron content. It is beneficial against many ailments, especially chronic fatigue, cancer, depression, and diabetes. Furthermore, beetroots contain vitamins A, E, C, B6, B12, and K.

Broccoli: It is beneficial for the human nervous system. It is rich in potassium, so it regulates blood pressure in humans. It also strengthens immunity due to the high contents of calcium and vitamin C.

Brussels Sprout: It contains the antioxidant alpha-lipoic acid (ALA). Owing to ALA, the glucose content in the blood of diabetic patients decreases (Salehi et al., 2019). Brussels sprout is rich in vitamin C and antioxidants. It also protects against viruses such as COVID-19 by supporting the immune system. It is known that low immunity increases the risk of contracting COVID-19. Therefore, Judit Molnár and Mahendra Pal mentioned Brussels sprout among immune-boosting vegetables (Molnar and Pal, 2021).

Turmeric and Ginger: Both are from the same family; curcumin in turmeric strengthens immunity. It is beneficial in treating many diseases such as influenza and respiratory tract infections. Furthermore, turmeric contains nutrients such as calcium, potassium, iron, thiamine, riboflavin, niacin, and ascorbic acid. Ginger, on the other hand, reduces cholesterol and relieves asthma, rheumatoid and intestinal tract infections of the immune system.

Spinach: Spinach and green leafy vegetables also contain plenty of vitamin K1. Spinach contains vitamins C and E, flavonoids, carotenoids, and antioxidants called phenolic compounds, which are good for respiratory tract infections, flu, and fatigue.

Cauliflower: It reduces mental fatigue by affecting the nervous system. Moreover, cauliflower is rich in vitamin C. Especially when consumed raw, more vitamin C is taken compared to oranges. It also contains folic acid and mineral salts. Enzymes found in vegetables, such as cauliflower, broccoli, and cabbage, are consumed by patients undergoing cancer treatment.

# 1.2. Meat Varieties, Dairy Products, and Eggs

At the beginning of the pandemic, the consumption of meat products decreased. In terms of food safety, it was believed that COVID-19 disease could be transmitted through meat, although it was not the definitive source during the pandemic.

Vitamins are compounds found in natural food sources, which help cell renewal and energy production, which the body needs in certain amounts, which need to be supplemented externally in cases where this amount cannot be supplied, and which act as regulators in different reactions in the body. Vitamin D deficiency may occur due to not going out for a while and not being exposed to the sun during the quarantine because sunlight is the best source of vitamin D. Vitamin D deficiency can be associated with epidemics, especially in winter. Vitamin D reduces the risk of death in respiratory tract infections and kills enveloped viruses while protecting the respiratory tract. Foods such as fish, liver, and egg yolk are rich in vitamin D. There are also milk and yogurt products with which vitamin D is taken (Alkhatib, 2020).

Antimicrobial peptides are evolutionarily conserved components of the innate immune system found in all classes of life. Antimicrobial peptides have also been shown to kill Gram-negative and Gram-positive bacteria, mycobacteria, encapsulated viruses, fungi, and even altered and cancerous cells. Antimicrobial peptides such as defensin, cathelicidin, and histadin have broad-spectrum antimicrobial activity against Gram-positive and Gramnegative bacteria, yeasts, fungi, and viruses. The general activation mechanisms of defensins and cathelicidins are explained as follows: the peptide containing a cationic charge binds to the outer surface of the pathogen by an electrostatic bond and then penetrates the cell contents by being embedded in the cytoplasmic membrane (Smet et al., 2005). It reduces viral replication rates through cathelicidins and defensins and prevents lung injuries that lead to pneumonia through anti-inflammatory cytokines. In this way, it strengthens the immune system. Another important component is zinc, which is found in red meat, poultry, fish, and especially oysters. Zinc helps maintain the body's ability to produce new cells and enzymes, process carbohydrates, fats and proteins, and increases the rate at which it heals muscles and wounds. According to some evidence, zinc helps prevent colds and viruses and minimizes allergy symptoms. Zinc is known to inhibit RNA-dependent RNA polymerase (RdRp) template binding of the Coronavirus (SARS) in Vero E6 cells (Muscogiuri et al., 2020).

Fish and other kinds of seafood have special importance in a healthy diet in terms of their rich mineral content. EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) have been demonstrated in studies to be critical for healthy fetal development, including neuronal, retinal, and immunological function. EPA and DHA may influence several aspects of cardiovascular function, including inflammation, peripheral artery disease, major coronary events. and anticoagulation. Fish are important sources of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). Omega 3 fatty acids are known to have an anti-inflammatory effect and are associated with mood. It is known that people with low fish consumption have a high risk of depression. Fish are also rich in antioxidants and tryptophan. It has been reported that fish consumption has a positive effect on respiratory diseases, and high-dose fish consumption is even inversely related to respiratory diseases such as asthma and chronic obstructive pulmonary disease (Kim and Hyunju, 2021). Another important component is the level of serotonin, which is

proportional to mood. The synthesis of serotonin depends on the presence of tryptophan. Sleep and appetite control depend on tryptophan, one of the functions of serotonin. Serotonin is found in fish, milk and dairy products, turkey meat, squid, and oysters. The brain produces serotonin from tryptophan, which is a kind of important neurotransmitter. It reduces stress and sleep disorders caused by the quarantine and makes individuals happy (Eskici, 2020).

On the other hand, meat, fish and poultry are the main sources of immunomodulatory amino acids and peptides and, therefore, have long been used as functional foods to treat respiratory diseases, fatigue and cold symptoms in the elderly, particularly in Asia (Alkhatib, 2020).

The intake of anserine and carnosine from high-protein meat, poultry, and seafood supports the immunological defense against infections caused by bacteria, viruses, fungi and parasites especially coronavirus, through enhanced immune cell functions of monocytes and macrophages. Meat products strengthen the immune system because they contain beta-glucan. Studies have shown that the antioxidant, anti-inflammatory, and antiviral properties of betaglucans play an important role in fighting against COVID-19. Many countries have approved beta-glucans as dietary supplements for a healthy lifestyle. Although the molecular pathways through which beta-glucans exert their protective and therapeutic properties have not been fully understood, research on the effects of beta-glucans on the immune system continues. Peptides are of animal origin, such as bovine blood, gelatin, meat, eggs, and fish. Peptides can be produced during the cheese ripening process. Peptide fractions can be obtained with anti-inflammatory activities and specific strains. Egg yolk and milk are rich in vitamins A and D, zinc and selenium, which are found in products such as cheese. Selenium is an important element of meat, chicken, fish, and eggs. Vitamin B12 enhances metabolism and is effective in the production of antibodies (Hamid et al., 2021).

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When scientists consider the egg with its high protein content, one of the most popular animal products, we can see how successful it was during the pandemic. There is increasing interest in dairy and egg-based diets with their immunomodulatory and anti-inflammatory properties attributed to certain chemical components.

Eggs contain iron, calcium, phosphorus, zinc, potassium, magnesium, sodium, and the majority of vitamins, with the exception of vitamin C. Riboflavin, niacin, thiamine, folate, and vitamins A, B6, B12, D, E, and K are among them. Eggs are a high-quality protein source that contains antioxidants similar to breast milk. Active biological compounds found in milk, including essential amino acids and fatty acids, make milk important due to its biological importance and value (Batiha et al., 2021).

According to the World Health Organization's healthy eating recommendations for adults during the COVID-19 pandemic, low-fat dairy products should be preferred instead of white meat and fatty dairy products instead of red meat [WHO, 2020].

Many compounds in meat varieties, dairy products, and eggs benefit human health and strengthen the immune system. If consumed in sufficient quantities, the side effects of the disease can be mildly avoided.

#### 1.3. Probiotics and COVID-19

Probiotics are microorganisms that are thought to positively affect health when consumed. During the COVID-19 pandemic, it was observed that the consumption of yogurt increased (Şahingöz et al., 2021). It is known that dairy products such as yogurt increase natural killer cell activity and reduce the risk of respiratory tract infections (Eskici, G., 2020). The demand for and the production of other dairy products, also known as probiotic foods, such as kefir and buttermilk, increased. Probiotics such as yogurt and kefir improve the microbiota of the gastrointestinal tract regarding the species of Lactobacilli and Bifidobacteria. In countries such as Turkey, Bulgaria, and Romania, the consumption of fermented milk has been observed to cause low mortality rates during the pandemic period. The reduced mortality rate is related to beneficial bacteria, amino acids, vitamins, enzymes, and minerals in products that should increase body resistance against COVID-19. Dairy products also have

therapeutic effects due to their antioxidant properties. They contain protective agents against viral infections such as COVID-19, SARS, and hepatitis A (Solak, 2020). Yogurt, a fermented product, can effectively reduce the symptoms of COVID-19 with multifunctional bioactive peptides. During the COVID-19 pandemic, yogurt has been researched more than other dairy products. Yogurt contains calcium, magnesium, potassium, zinc, and vitamin B. Surprisingly, yogurt contains more protein, minerals, and vitamins than milk. Furthermore, the beneficial bacteria in yogurts strengthen the intestinal barriers by increasing cell immunity, reducing oxidative stress, and preventing inflammation. Therefore, it plays an effective and important role in the management of COVID-19. Probiotic strains involved in milk fermentation contribute to the formation of oligopeptides that can act as bioactive peptides. Additionally, digestion by pepsin and trypsin increases the production rate of such products in the human body. Consumption of probiotics and fermented dairy products significantly reduces respiratory tract infections (Gouda et al., 2021). Protection from viral infection has been shown as a benefit of probiotics. Probiotics exert their beneficial effects on the host immune system through the modulation of the immune response, maintaining intestinal homeostasis, and producing interferon, thereby suppressing the development of the virus (Sundararaman et al., 2020). According to the data obtained from all these studies, it is undeniable that the use of probiotics in COVID-19 is important in reducing the burden and severity of the pandemic. Their effect on cytokine production by epithelial cells in the intestines increases immunity, affects IgA secretion. phagocytosis and macrophage activation. and systemic inflammation. The studies have investigated the potential role of probiotics in alleviating COVID-19 through direct interaction with the intestines or lungs. These studies have concluded that more research must be done (Bottari et al., 2021).

Some antiviral, anti-inflammatory, and antimalarial drugs have been used to treat COVID-19. However, none of these drugs have been approved as effective curative treatments for COVID-19. Probiotics and prebiotics can also be used as an alternative to these drugs or different applications to fight against COVID-19 (Olaimat et al., 2020).

Probiotics can help prevent COVID-19 by protecting the lung microbiota because they play an important role in people's susceptibility to infectious diseases (Olaimat et al., 2020). Therefore, there is a need for in vitro and clinical studies to examine the potential protective and curative effects of probiotics on SARS-CoV2 infection. Curd is a traditional yogurt or fermented

milk product from the Indian subcontinent, usually made from cow's milk and sometimes from buffalo's or goat's milk. Probiotics such as curd are an excellent source of maintaining the composition and function of gut bacteria important for the immune system. They are a source of beneficial bacteria (probiotics and lactobacilli) for the immune system to keep up with infections and viruses, and they strengthen the immune system. Curd is popular throughout the Indian subcontinent (Caballero et al., 2015). With the regular consumption of "curd," cholesterol levels in the human body decrease and the risk of hypertension and blood pressure problems is reduced, thus keeping the heart and cholesterol levels stable (Ahmad and Ghosh, 2020).

#### 1.4. Herbs and Oils

Fats are among the essential nutrients. Chemically, they are also known as triglycerides. Fats are macromolecules taken into the human body with various foods (Wikipedia, 2022). According to their source, oils can be classified as vegetable oils and animal fats (Wikipedia, 2022).

# 1.4.1. Herbs

Herbs are eukaryotic creatures that are widely used in the medical and spiritual fields and as edibles in the kitchen. Structurally, they are salty and aromatic plants. In plants used in the medical field, any plant parts such as leaves, roots, flowers, and seeds can be used (Wikipedia, 2022).

Studies and research have also been carried out on natural products to make a contribution to the treatment of COVID-19, the development of the immune system, and the reduction in its transmission (Ekici, 2021).

Examples of natural products are plants and herbs used especially in the medical field. Many investigations have revealed that biochemical and bioreactive substances in plants can treat viral diseases and prevent the occurrence of these diseases (Dhama et al., 2019). Throughout history, plants have been used to treat various diseases in traditional medicine. Although plants have been used in the treatment of diseases since ancient times, recent scientific evidence has accelerated the therapeutic effects and usefulness of herbs (Dhama et al., 2019). Over time, the structure and content of many plants have been examined, their metabolites have been scanned, and plants have been subjected to various tests to determine whether they exhibit antiviral properties. These tests are clinical tests that include many processes, from understanding the therapeutic structure of plants to the isolation of active substances. As a result of all these studies, it has been determined that plants can be effective in the medical treatment of viral diseases (Dhama et al., 2019).

*Glycyrrhiza glabra L.* (Fabaceae - Licorice/Licorice, sweetwood): It is commonly known as licorice, sweetwood, or mulaithi. It is a small perennial herb that grows in Eurasia, North Africa, and West Asia. The plant of the genus Glycyrrhiza is spread worldwide, and there are more than 30 species (Batiha et al., 2020). Glycyrrhizin is the substance that is found in this herb's structure, activates immune system functions, and has antiviral effects. It has been found that the prevents SARS-CoV-1. According to a study, glycyrrhizin stimulates and activates lymphocytes. It has also been recommended to use glycyrrhizin to relieve acute respiratory problems. In China, glycyrrhizin is used as an adjuvant (Fiore et al., 2008; Bailey et al., 2020) in the treatment of COVID-19. It should not be used together with drugs containing licorice and glycyrrhizin, such as diuretics, cardiac glycosides, antihypertensives, corticosteroids, and stimulant laxatives (Özay, 2022).

Allium sativum L. (Liliaceae - Garlic/Garlic): It has been reported that since the garlic extract has an immunomodulatory effect, the leptin increase induced by the decrease in the macrophage levels of T cells caused by COVID-19 can be prevented. Garlic supplementation can increase CD4+ and CD8+ cells (Özay, 2022).

In a model study, three hundred eighteen phytochemicals were obtained from 11 plants. In this study, the plants' phytochemicals were subjected to insertion against major protease and ACE2 targets. This study determined that plants named *Piper longum* L., *Phaseolus vulgaris* L., *Curcuma longa*, *Ocimum gratissimum* L., *Syzygium aromaticum*, *Artemisia abstinidium* L., and *Inula helenium* L. had lower binding energies against the receptors and were responsible for COVID-19 infections. It was suggested that they could be used against COVID-19 (Joshi et al., 2020).

In a study, diallyl disulfide in garlic, curcumin in turmeric, capsaicin in pepper and limonene in cardamom were tested by the molecular docking method, and their metabolites were examined to reveal which was the best medicine against COVID-19. Accordingly, it was determined that curcumin might be the best medicine due to its active component (Mohammedi, Shaghagi, 2020). In addition to their strong antioxidant and antimicrobial activity, labdanum (*Cistus* spp.), olive leaf (*Olea europea L.*), and some thyme species (Origanum, Thymus, and Thymbra), which have high antiviral activity and the ability to strengthen the immune system, can be used to treat COVID-19 (Şekeroğlu and Gezici, 2020). Furthermore, *Artemisia kermanensi*,

*Eucalyptus caesia, Mentha* spp., *Rosmarinus officinalis, Satureja hortensis,* and *Zataria multiflora* are plants with high phenolic content so that they can prevent coronavirus proliferation. Additionally, studies have shown that biflavonoids in *Torreya nucifera* inhibit SARS-CoV 3CL pro replication (Hefferon et al., 2021).

# 1.4.2. Oils

Malnutrition can lower the immune system. Bioactive compounds can strengthen the immune system. In reducing the effects of COVID-19, the substances in oils also have effects on immunity and the disease. Omega-3 PUFA-containing oils are among them (Özsoy and Saka, 2021).

Inflammatory cells are affected by the fatty acid composition in oils. Changes occur in the PUFA ratio of cells because the fatty acid composition of cells affects their functions. Long-chain fatty acids affect inflammation through various mechanisms. Among the important fatty acids, eicosanoids derived from arachidonic acid help to develop an inflammatory response. Omega-3 PUFA shows the fatty acid with the strongest immodulatory effect. Natural and acquired immunity can be affected by this fatty acid. Fish oil contains EPA and DHA. As the consumption of foods containing fish oil increases, the arachidonic acid in cell membranes is slightly replaced by EPA. As EPA and DHA increase and arachidonic acid values decrease, it causes a decrease in components with inflammatory effects. Omega-3 PUFA diet enrichment reduces the production of proinflammatory cytokines by monocyte and neutrophil chemotaxis (Özsoy and Saka, 2021).

When a person is infected with COVID-19, the immune cells in the body and the cytokines they contain can cause a cytokine storm in the immune system. This is an extreme immune reaction. The inflammatory chemicals caused by COVID-19 force immune cells to destroy them. This inflammatory response is related to the severity of COVID-19 in patients (Pamukçu, 2020). In addition to strengthening immunity with drugs, nutrition is also important. Oils can also help strengthen immunity with the compounds in their content. Coconut oil, especially virgin coconut oil, has anti-inflammatory and antiviral properties (Tandaa et al., 2021).

# 1.5. Grains

Grains are the important nutrients in human nutrition. Grains are stored before being presented to the consumer after being harvested due to economic reasons. Grains consist mainly of carbohydrates, which means they are a source of energy. Moreover, cereals contain protein, e.g., there is 8% protein in bread and 10% in bulgur. There are vitamins and minerals such as vitamin E, quercetin, selenium, copper, phosphorus, iron, magnesium, zinc, vitamins B1 (thiamine) and B2 (riboflavin), niacin, vitamin B6, and folate in cereals. Whole grain intake is recommended as 25 grams per day for women and 38 grams for men. Consuming whole-grain foods regulates the digestive system by preventing its deterioration. Consuming a high amount of grains protects against many diseases such as cardiovascular diseases, type 2 diabetes, and cancer (Tapan, 2021). Grain varieties such as oats, quinoa, buckwheat, rye, millet, and bulgur support the intake of fiber into the body. Fibers taken into the body strengthen the immune system. With the onset of the COVID-19 outbreak, individuals who remained closed in their homes increased the consumption of homemade foods and consumed foods such as homemade pizza and bread (Hero et al., 2022). Consuming whole grains or increasing the consumption rate has an anti-inflammatory effect on COVID-19 (Daziroğlu et al., 2021). The dietary fiber in whole grains supports the immune system and provides an anti-inflammatory effect. It ensures the formation of short-chain fatty acids (SCFAs). For example, they allow the proliferation of bacteria such as bifidobacterium and lactobacillus (Topuz, 2020). Beta-glucan is the name of the nutrient found in grains as a source of fiber. Beta-glucan is naturally found in the cell walls of cereal foods such as barley, millet, rye, and oats. It is thought to protect SARS-CoV-2 by activating the protective bacteria in the body. Therefore, it is recommended to take about 500 mg of beta-glucan daily (Tapan, 2021). It helps to reduce cholesterol, increase heart health, regulate blood sugar levels, and activate the protective bacteria against COVID-19 by stimulating the immune system.

# 1.5.1 Some Important Grain Types

Wheat <u>(*Triticum aestivum*)</u>: It is a cultural product that dates back 12 thousand years. It constitutes 19% of the daily calories and 21% of the protein that people take. There are two types of wheat. Of wheat, 95% is bread wheat (*Triticum aestivum* L.), and 5% is durum wheat (*Triticum durum* Desf.). Wheat flour contains fiber, phytochemicals, vitamins, and minerals. Whole wheat is good for cardiovascular diseases, cancer, type 2 diabetes, and obesity (Kılıç, 2021).

Siyez Wheat <u>(*Triticum monococcum*)</u>: It is considered the ancestor of wheat. There are elements such as fatty acids, fructan, zinc, and iron in einkorn wheat, and it is a protein source. It contains bioactive components such as

carotenoids, conjugated phenolics, tocols, and polyphenols. These components have an anti-inflammatory effect. They allow the proliferation of pro-inflammatory cytokines.

Oat (<u>Avena sativa L</u>.): It has a culturally more recent history. While it was used as fodder for animals such as horses and donkeys in the past, it has started to be used in human nutrition nowadays due to the vitamins, minerals, fiber, protein, fatty acids, and phytochemicals in its content. Despite its use as a dietary product, it is also consumed in varieties such as baby food, oat soup, and biscuits. The amount of raw cellulose is higher than in other cereals. In a study by Pameranz (1986), oats had the most B and E vitamin complexes. Vitamins are located in the embryo and aleurone part of the bran part. Oats are thought to be good for many diseases such as cancer and cardiovascular diseases, especially type 2 diabetes and celiac disease.

Millet or Corn: There are many types of corn. The most well-known ones in Turkey are pearl millet (*Pennisetum glaucum*) and ragi millet (finger millet) (*Eleusine coracana*). According to a study by Boz (2014), millet, known as finger or ragi millet, contains more calcium than wheat. Moreover, the millet variety, known as sand millet, has more protein and phenolic substances than any other grain. Therefore, it is good for chronic diseases such as cancer, cardiovascular diseases, and type 2 diabetes. The phenolic content in corn varies according to the variety and size of corn. Additionally, the phenolic structures in corn are also different.

Rice: The rice grain consists of pericarp, husk, seed coat, aleurone layer, endosperm, and embryo. Among cereals, the highest carbohydrate content belongs to rice. Rice contains vitamin B, phosphorus, selenium, potassium, manganese, and minerals. Rice flour is recommended for infant feeding. It is known to be effective in the fight against cardiovascular diseases, celiac disease, and cancer.

Barley: Nowadays, it is used in bread making by being turned into flour. It is the grain richest in niacin. In terms of minerals, it comes after oats among cereals. It supports immunity against many diseases. Therefore, it is beneficial against cancer, type 2 diabetes, and cardiovascular diseases.

Rye: Rye is used by being turned into flour today. Dough is made with whole wheat flour due to its low viscosity. Since the bran part is consumed with flour, it is rich in vitamin B. It has especially high riboflavin content. Rye is thought to be good for many diseases such as cancer, cardiovascular diseases, type 2 diabetes, and celiac disease.

### 1.6. Vitamins / Minerals and COVID-19

Vitamins and minerals have also been an important research topic during the pandemic. Scientists have questioned how vitamins and minerals affect the process in bodies. Studies have shown that foods are rich in vitamin A, vitamin B varieties, vitamins C, D, E, and K, sodium, potassium, phosphorus, magnesium, calcium, zinc, copper, iron, selenium, manganese, cobalt, sulfur, and iodine.

### 1.6.1 Vitamins

Vitamin A: It is usually found in foods such as meat, fish, and eggs. Carotenoids in the form of carotene are mostly found in fruits and vegetables. The oxidative ability of vitamin A has been the subject of research. It has both antioxidant and prooxidative properties. It is an immune regulator and good for respiratory diseases. It is known that coronavirus affects the respiratory tract. Therefore, vitamin A provides examples that prove its usefulness. It increases cytotoxicity and takes part in T-cell proliferation (Jovic et al., 2020).

# **Types of Vitamin B**

Vitamin B1: It is also known as thiamine and is an essential cofactor in the tricarboxylic acid cycle. It has antioxidant properties and reduces the risk of death when taken in high doses together with vitamin C. Thiamine has been documented to play an important role in eradicating the SARS-CoV-2 virus by triggering humoral and cell-mediated immunity. Therefore, adequate levels of thiamine help build immunity against SARS-CoV-2 patients (Shakoor et al., 2020),

Vitamin B2: It is also known as riboflavin and is an important factor for the immune system. It has immunomodulatory effects, and its deficiency regulates proinflammatory gene expression. It reduces the possibility of contracting the disease (Dehghani-Samani et al., 2020).

Vitamin B3: Vitamin B3, known as niacin, is used as a coenzyme in metabolic processes. Nicotinamide has a potential binding affinity for Sars-CoV-2 protease. Niacin also acts as an anti-inflammatory agent, reducing neutrophil swelling in ventilator-induced lung injury patients (Nagai et al., 1994). Jovic et al. (2020) stated in their study that nicotinamide reduced viral infection and stimulated defense mechanisms. Given the therapeutic properties of niacin, it can be used as an adjuvant in treating COVID-19 patients.

Vitamin B5: It is also known as pantothenic acid and is highly available in plants and animals. It has inflammatory properties, facilitates the healing of wounds, and reduces inflammation (Peterson et al., 2020).

Vitamin B6: It is also known as pyridoxine and is involved in reactions related to antibodies and in the immune system (Parra et al., 2018). A recent study revealed that pyridoxine supplementation helped alleviate COVID-19 symptoms by improving immune responses, reducing pro-inflammatory cytokines, promoting endothelial integrity, and preventing hypercoagulation (Mikkelsen and Apostolopoulos, 2019).

Vitamin B9: It is also called folate and plays important and complementary roles in both innate and adaptive immune responses and has accepted health claims in the European Union since it contributes to the normal function of the immune system. It is known that vitamin B9 deficiency causes megaloblastic anemia, growth retardation, and proinflammatory cytokine profile in infections due to combined immunodeficiency (Serseg et al., 2021), which are problems that can be reversed with folate therapy. It can be used in the early stages of the disease to overcome respiratory problems and relieve symptoms.

Vitamin B12: It is also called cobalamin and plays an important role in regulating immunity and contributes to intestinal barrier function. It protects the body against respiratory tract infections and other infections. It is found in animal-based foods such as liver, meat, fish, eggs, milk, cheese, and yogurt (Kandeel and Al-Nazawi, 2020).

Vitamin C: It is also known as ascorbic acid and has antiviral, antioxidant, and anti-inflammatory properties. Vitamin C strengthens the immune system, makes it easier to fight the disease, and reduces the risks of upper respiratory tract infections (Senses, 2020).

Vitamin D: Vitamin D is mostly found in oily fish and egg yolk. In addition to its antimicrobial effects, it protects cells against bacterial infection as a protective barrier. Moreover, vitamin D strengthens the immune system and reduces the risks caused by upper respiratory tract diseases (Huang et al., 2020).

Vitamin E: It is an effective antioxidant and has anti-inflammatory properties. Vitamin E, secreted in the liver, participates in metabolism by the intestines and supports the immune system (Hamada, 2020).

Vitamin K: Vitamin K is a cofactor and coenzyme. It is used in the synthesis of proteins and other physiological functions. Vitamin K provides

blood coagulation, thus reducing the risk of bleeding and the severity of COVID-19 and the risk of complications (Janssen and Walk, 2020).

# 1.6.2. Minerals

Sodium: It plays an important role in electrolytic balance and suppresses SARS-CoV2. The research demonstrated a significant decrease in the sodium concentration of patients. At the same time, it was stated that the decrease in sodium increased the disease severity (Lippi et al., 2020).

Potassium: It lowers the risk of heart diseases and ensures that the lung is not damaged. Moreover, potassium reduces the symptoms of COVID-19 (Lippi et al., 2020).

Phosphorus: It creates protein for the maintenance, repair, and growth of cells and tissues. A low concentration of phosphorus leads to problems in the immune system caused by COVID-19, and the damage caused by the disease to cells and tissues is not repaired (Ni et al., 2020).

Magnesium: It plays a role in the immune system and has various functions such as adhesion of immune cells, synthesis of immunoglobulin, binding of IgM to lymphocytes, and cytolysis depending on antibodies. Studies have shown that when magnesium is used together with vitamins D and B12, the rate of progression can be reduced in elderly patients with COVID-19 (Ni et al., 2020).

Calcium: Calcium strengthens our bones and allows viruses to be separated from cells. It has been revealed to protect cells in diseases similar to colds. The severity of the disease is inversely proportional to the ratio of calcium in patients' bodies (Kum et al., 2021).

Zinc: Zinc is found in the circulatory, reproductive, and nervous systems and has antiviral properties. It has been stated that SARS coronavirus inhibits RNA polymerase (Zhang et al., 2018).

Copper: It is an essential micronutrient against viral infections. Its absorption takes place in the small intestine. It carries out many processes related to maintaining the body's ionic balance. Copper produces antibodies against pathogens and has important functions in the immune system. Therefore, it may be a good option to take or supplement copper during the COVID-19 outbreak (Raha et al., 2020).

Iron: Iron plays an important role in producing hemoglobin and red blood cells. SARS-CoV-2 induces ferritin production in conjunction with the rapid onset of inflammation in the acute phase of infection. Therefore, the immunomodulatory effects of ferritin contribute to generating reactive oxygen species (ROS) and lead to tissue damage. In contrast, iron chelation therapy is a novel approach to COVID-19. Iron chelation therapy is the most effective approach in a wide range of diseases associated with iron overload. Therefore, iron chelation therapy is considered a viable approach to improve survival in COVID-19 patients (Kumar et al., 2021).

Selenium: It has antiviral and anti-inflammatory properties. Selenium regulates the immune system and reduces the severity of symptoms caused by COVID-19 (Zhang et al., 2020b).

Manganese: It is responsible for antioxidant activity and energy production after amino acid breakdown. Manganese is used in treating COVID-19 because it regulates the immune system and has antiviral properties (Haase, 2018).

Cobalt: Cobalt is responsible for the maintenance of the nervous system and the production of red blood cells (Kumar et al., 2021).

Sulfur: The biological function of sulfur is to produce essential amino acids such as cysteine and methionine. Additionally, it also takes part in biocatalytic processes, cell membrane transfers, immune system, and blood coagulation. Due to its multiple therapeutic roles and involvement in the respiratory system, sulfur may exert a protective effect against COVID-19 (Kumar et al., 2021).

Iodine: Iodine is responsible for producing thyroid hormone. Therefore, it plays an important role in metabolism and has antiviral properties. When taken in high doses, it reduces the severity of respiratory diseases (Kumar et al., 2021).

#### 2. CONCLUSION

Foods are the basic structures we need to sustain our lives. They contain many valuable bioactive compounds while protecting our body against different diseases. COVID-19 has recently become the most important among these diseases. This review discussed and evaluated the relationship between foods and COVID-19. In this way, the potential foods that can be used to fight against COVID-19 symptoms are presented here. Since COVID-19 is a new epidemic, the certainty of the effects of the nutrients mentioned in the article is still the subject of research. In the future, conclusive results about the effects of food on COVID-19 will be obtained with more emphasis on this issue.

As a result, the struggle of humanity against COVID-19 continues. Apart from drugs, immunity can be increased and the harmful effects of COVID-19 can be alleviated with proper nutrition. We need to be aware of the contents of the foods we consume. Our diet plays a big role in the pandemic process. It is necessary to consume fruits, vegetables, grains, meat, milk, oil, various plants, vitamins, and minerals in the correct and sufficient amount. We think this pandemic will be overcome more easily and in a shorter time in this way, which will be supported by research.

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# **CHAPTER 14**

# WALNUT FRUIT: A GREAT MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR BIODIVERSITY IN TURKEY AND IN THE WORLD

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### **INTRODUCTION**

The plant diversity has been used frequently in recent years as a valuable resource in agriculture, industry, medicine and biotechnology as insurance of future life of humanity. For agriculture, plant biodiversity in an important source because it provides raw material for most of the agriculture area. In terms of breeding, this kind of agricultural biodiversity is vital because breeders need rich gene combinations that produce the plant varieties upon which agriculture depends.

Plant biodiversity is important in both horticultural crops and genetic resources. They can be used in traditional farming systems, conventional or modern breeding or genetic engineering. Thus, there were a strong link between horticultural plant biodiversity and horticultural genetic resources (Keles et al., 2014; Guney et al., 2021).

Approximately 90% of the world's population use mainly 15 different plant crops for foods. Not only cultivated but also wild relatives of horticultural crops also make an important contribution to the field of medicine, agriculture, industry etc. They are accepted an important natural resource, which have an indispensable place in use in different areas including agriculture. Wild plants originate half of the world and approximately 80% of the world's population uses wild plants as the primary source of medicines. Similarly, in medicine, nearly 30% of drugs are developed from plants. Wild relatives are more resistant to various diseases and pests, with wide adaptation capacity, in order to increase needs to be cultivated (Orhan et al., 2020) More recently plant diversity accepted as vital source in agricultural biotechnology applications. Thus, the plant diversity rich countries had advantages in different sectors including agricultural biotechnology (Bozhuyuk et al., 2020).

Turkey is among the countries with rich horticultural plant biodiversity and one of the most important gene centers for some of fruit species. The geographical position of Turkey offers a suitable position for the cultivation of many fruit species including Persian walnut (*Juglans regia* L.) (Ercisli et al., 2012). Turkey is a bridge between the continents of Asia and Europe. Therefore, throughout the history of humanity, between east and west, north and south all mass movements due to trade, migration, war occurred on Anatolia. During these mass movements for various reasons, the people passing by took the seeds of every kind of fruit that they brought with them and consumed and finally they left seeds here. These seeds are main source of tremendous variability. In additions, the country surrounded by three main sea (Mediterranean, Aegean and Black sea) which also promote different climate formation. In particular a great morphological, biochemical and molecular richness of fruit species are evident in Turkey and this kind of diversity cannot be seen any other countries. Therefore, Turkey accepted a fruit-growing paradise (Keles et al., 2014; Orhan et al., 2020).

The fact that walnut cultivation is widespread in Anatolia and Central Asia due to several reasons. The most important reason is its long-term storage capacity which easily found year around, second one is that walnut fruits important among the diets of people living in these countries. Therefore, it has been an important part of the daily diet of mankind since its creation (Amaral et al., 2003).

Walnut (Juglans regia L.) is belonging to Juglans genus. Within the genus Juglans, 18 walnut species whose characteristics have been determined today are known. Walnut species whose properties are known today are: Juglans regia L. Juglans australis Grisebach, Juglans boliviana Dode, Juglans californica S. Wats, Juglans cathayensis Dode, Juglans cinerea L., Juglans ailantifolia Carr, Juglans ailantifolia coriformis (Max) Reh., Juglans hindsii Jeps, Juglans jamaciensis C.D.C, Juglans major (Torr) Heller, Juglans mandshurica Maxim, Juglans microcarpa Berlandier, Juglans mollis Engeim, Juglans nigra L., Juglans olanchana Standley and Williams, Juglans stenacarpa Maxim, Juglans sieboldiana (Bernard et al., 2018). Among these species, it is the first thing that comes to mind when walnut is mentioned with its superior fruit quality.

Anatolian walnut, also called Persian walnut and English walnut, is *Juglans regia* L. Turkey is the homeland of walnuts and natural spreading areas. In Turkey, other than *J. regia* L. walnut species do not occur naturally. *J. nigra, J. hindsii* and *J. cinerea* are the other important *Juglans* species. However, these species are important for timber production and use of their fruits are very limited.

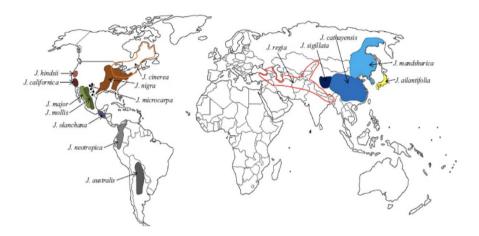


Figure 1. Distribution of Juglans species in the world (Bernard et al., 2018).

Walnut is one of the fruit trees naturally found most of the regions of Turkey and people use it for centuries for its highly nutritional fruits. In addition, walnut timber very valuable for furniture industry in Turkey (Ozcagiran et al., 2007). Walnut (Juglans regia L.) is the most important nut crop grown in Turkey. The number of walnut trees in that country is estimated to be more than 11 million, mostly seedlings, distributed on borders of farms and orchards or expanded in valleys. Total production of inshell walnut is estimated at 290,000 t, and Turkey is considered the fourth largest walnut producer in the world. Use of walnut seedling trees throughout the centuries has provided unique opportunities for breeders to select promising genotypes (Ercisli et al., 2012). Continuous seed propagation for thousands of years in Turkey has given rise to a great number of seedling walnut trees, which represent valuable walnut genetic resources. The number of native trees is estimated to be over 5 million and they possess large genetic variability in yield, nut and kernel characteristics, late bud breaking, late flowering, winter hardiness, tolerance to disease etc. (Orhan et al., 2020). This high genetic variation of native walnut populations throughout country presents good opportunities for walnut breeding to obtain new cultivars directly or to use them in cross breeding activity.

In Turkey a large number of selections studies done on seed propagated walnuts and the aim of walnut selection studies is not only about obtaining walnuts with good fruit quality, but also to obtain genotypes with fruiting on lateral branches. Another parameter considering selection is resistance to diseases.

## **1. MORPHOLOGICAL MARKERS**

The main diversity sources of walnut trees are coming firstly from its out-crossing nature and secondly traditional simple seed propagation technique applied by in particular central Asian countries. Thus, in those countries a tremendous morphological variation with respect to different tree and fruit characteristics such as time of male and female flowering, harvest date, tree growth habit, vigour, the density of branches, bud break, lateral leafing, dichogamy, fruit size, fruit and kernel color, fruit shape, shell thickness, kernel ratio etc. are evident (Botu et al., 2007; Ercisli et al., 2012; Khadivi-Khub, 2014; Lotfi et al., 2019; Mahmoodi et al., 2019; Bujdoso and Cseke, 2021; Cosmulescu and Ionescu, 2021; Shah et al., 2021).

Above morphological parameters have been considering routinely in walnut diversity studies for a long time. However, more recently different morphological traits including inflorescence, the abundance of male and female inflorescences, fruiting characteristics, kernel removal from nuts etc. have also been using by different researchers as morphological parameter (Poggeti et al., 2017; Rezaei et al., 2018; Shah et al., 2021).

The variability in vigour reported in plants is due to the heterozygous nature of walnut seedling trees (Solar and Stamper, 2006, Zeneli et al., 2005) that can be explored for developing low vigour genotypes for tree size control in walnut (Rezaei et al., 2018). As walnut produced mainly for fruit consumption, fruit (nut) and kernel characteristics are essential to identify walnut genotypes for commercial cultivation (Ebrahimi et al., 2011; Karimi et al., 2014; Poggettia et al., 2017; Bernard et al., 2018; Shah et al., 2021). The literature on morphological traits of walnuts shows that the walnuts are highly diverse in all the growing regions in the world (Akhiani et al., 2017; Zare-Rashnoodi et al., 2017, Khadivi et al., 2019; Shah et al., 2021).

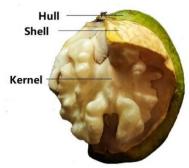
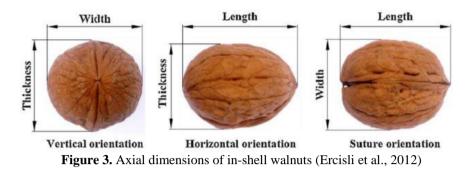


Figure 2. Structure and components of in-hull walnuts (Ercisli et al., 2012)

Modern objectives in plant breeding may be achieved by the evaluation of traits amongst genetic resources and combination of those in one cultivar. For walnut there were a large number of morphological markers which could be easily applicable on germplasm and might be appropriate for morphological classification Thus, morphological traits are the vital for parents in breeding and also is the first choice used for describing and classifying the walnut germplasm all over the world (Norouzi et al., 2013; Jacimovic et al., 2020). Morphological parameters could be accompanied with some sophisticated statistical methods including heat maps, principal components or cluster analysis and can be used as useful tools for screening the accessions. In addition, morphological characteristics sometimes have correlation or are associated with characteristics that are difficult to evaluate such as disease susceptibility. Therefore, they may be useful as markers in breeding programs (Jacimovic et al., 2020). The main morphological parameters (markers) for walnut cultivars evaluated in morphological classification studies are:

Tree growth habit
Late leafing
Lateral bud fruitfulness
Dichogamy
Fruit (nut) weight
Nut shape
Kernel weight
Kernel ratio
Kernel color
Shell thicknes



In Turkey, most of the standart walnut cultivars have early leafing and low lateral bud fruitfulness characteristics. However, studies are also showed that among seed propagated walnut genotypes there were late leafing and lateral bud fruitfulness genotypes which is important for sustainable walnut cultivation in Turkey (Keles et al., 2014). In walnut breeding studies main aim is to obtain cultivars which shows high nut quality, resistance to diseases, late leafing and lateral bud fruitfulness (Mitrovic et al., 2007). High yielding capacity is also important for walnuts and the lateral fruitfulness strongly affects and determined the potential yield in *J. regia*. The main biotic and abiotic environmental problems in walnut cultivation is walnut blight and anthracnose and spring frost. Late leafing is important to avoid spring frost.

Morphological markers form the basis of breeding studies (in particular cross breeding and selection). It is not possible out breeding studies without using morphological markers.

Morphological markers widely used cultivar or genotypic identification in walnut. The main advantage of morphological markers is their easy observability. However, main problem with morphological markers are they strongly affected by environment, their phenotypic traits cannot be reflected by their genetic traits, insufficient variation, not appropriate growth of the desired phenotypic traits and long time of emergence, respectively. All those take disadvantage for plant breeders. Although, morphological markers have bottlenecks for walnuts but these form an essential component to assess diversity and to identify a genotype with superior economic traits.

Considering native walnut growing areas around the world including China, Kyrgyzstan, Turkmenistan, Uzbekistan, North-western Himalayan regions of India, Pakistan, Nepal, Afghanistan, Iran, Azerbaijan, Armenia, Georgia, and Turkey (Aradhya et al., 2010), *J. regia* native populations possess tremendous variation in morphological traits including tree growth habit, fruit shape, fruit size, kernel color, kernel percentage, shell thickness etc.). This tremendous variation is vital for breeding activities to obtain walnut cultivars with different fruit characteristics. In particular in those countries walnuts mostly propagated from seeds and each tree exhibit different traits and farmers selections conducted centuries in those areas have significantly contributed in selecting the genotypes with late flowering, disease resistance, kernel quality, and genotypes adapted to broader geographic conditions under current extreme climate change scenarios.

The rich walnut genetic resources offer a great chance to researchers and the best strategy to achieve the modern objectives in walnut breeding could be evaluate the available genetic resources widely found among seed propagated materials which have superior morphological traits and then use them into conventional breeding to combine these in one cultivar (Pop et al., 2013; Akca et al., 2020). In fact, seed propagated walnut trees is ready material to start cross breeding and screening of such resources for different traits will produce vast information for researchers and scientists to identify valuable walnut genetic resources (Hassani et al., 2020; Guney et al., 2021). Furthermore, to better understand the plant genetic resources, different methods are evaluated and adopted to improve the collection, conservation, and germplasm improvement to broaden the walnut's genetic base. For collection and conservation of promising genotypes, various morphological traits have been adopted by different researchers, such as dichogamy (Ebrahimi et al., 2015), spring frost resistance (Khadivi et al., 2019), fruit traits (Ahandani et al., 2014; Cosmulescu and Stefanescu, 2018) and are used in breeding programmes for the improvement of new walnut cultivars (Francesca et al., 2010).

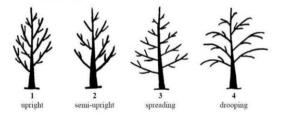


Figure 4. Tree growth habit in walnuts

### 2. BIOCHEMICAL MARKERS

The main problem with biochemical markers in fruit species is that there are not enough biochemical markers. As with morphological markers, biochemical markers are also strongly influenced by the environment, which is a weakness in the use of biochemical markers. However, this effect may disappear when cultivars or genotypes are found in the same ecological conditions.

The main biochemical parameters (markers) for walnut cultivars evaluated in biochemical classification studies are:

-Oil
-Protein
-Ash
-Fatty acid composition
-Tocopherols
-Antioxidant capacity
-Total phenolic content
-Phenolic acids
-Amino acids
-Dietary fiber
-Antioxidant enzymes (SOD, CAT, GSH-Px)
-Isoenzymes

More recently there is tendency among consumers to functional and health foods. Among fruits, walnut fruits well characterized by high level of fat, protein, tocopherols and antioxidants (phytosterol and polyphenols). In addition, the fruits are also having high minerals including iron, zinc, copper, magnesium and phosphorus (Unver and Sakar, 2011).

For centuries because of rich nutrition content, walnut fruits play an important role in the Mediterranean diet (Ros, 2010). The fruit is high storable capacity and thus can be found year around on markets and widely used in food, pharmaceutical and cosmetic industries (Ercisli et al., 2012). Thus, its production amount and growing areas in walnut producing countries is constantly increasing and becomes the second-largest nut after almond in terms of production amount (FAO, 2020).

In recent years, studies involving substances with high biochemical content and higher functional properties in terms of human health have accelerated in breeding studies and these studies are evaluated in the special breeding category. Walnut is also one of the fruits evaluated in this respect (Ada et al., 2021).

Considering biochemical content of walnut fruits, it has a high nutritional value as they contain high fat and protein. The most important feature of walnut oil is that it is very rich in unsaturated fatty acids. The high amount of linoleic acid in walnut oil makes walnuts a unique food. In addition, high biological quality protein, vitamins and minerals in its composition increase its nutritional value. Recent epidemiological studies show that walnut consumption reduces cardiovascular diseases. It is stated that this is due to the antioxidant properties of polyphenols abundant in walnut fruits (Bacerra-Tomas et al., 2019).

As biochemical markers especially the isozymes and allozymes were used to infer the walnut diversity. The iso-enzymes analysis (Vyas et al., 2003, Foroni et al., 2005; Zeneli et al., 2005) are rapid methods to assess diversity and requires a small amount of tissue for analysis, but these have a limited application in genetic diversity analysis of walnut because of their limited number and low resolution of markers combined with sensitivity to environmental factors (Kumar, 1999).

### **3. MOLECULAR MARKERS**

As indicated before both morphological and biochemical markers are inadequate due to less differentiating traits and being influenced by environmental factors, which led to development of DNA based markers. During last four decades molecular markers were gained more importance for efficiently use in breeding programs for different fruit crops. Compared to previous markers, molecular markers are more objectively expressing genomic traits because they are stable, detectable in all tissues irrespective of growth, development and differentiation, and also remain unaffected by fluctuations in environmental conditions and cultural impacts as well. The all markers have their own strengths and weaknesses based on their selection and aim of study. They used to detect variations in DNA sequences of varieties, cultivars, accessions, genotypes, parents or individual plants used in crop improvement programs. The molecular markers are important element of plant biotechnology applications because they determine genomes' structure and the genetic mechanism behind economically important traits. They used in many different areas including genetic diversity, genetic relationships, taxonomy, phylogenetic analysis etc. (Shah et al., 2020). Classification of different molecular markers used in fruit crops is illustrated in Figure 5.

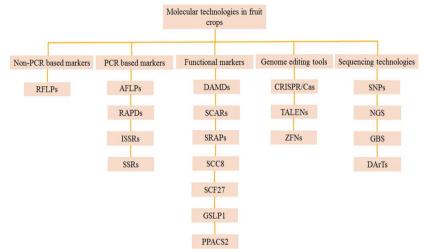


Figure 5. Classifications of different molecular markers

The characteristics of different molecular markers used in plant science should be polymorphic, co-dominant inheritance, easy to access, frequent occurrence in genome, require little amounts of DNA sample and easy exchange of data among laboratories (Orhan et al., 2020). There are different kind of molecular markers used in plant science vary from each other due to following features, i.e., genomic richness, detection level of polymorphism, specificity of locus, reproducibility and costs assay (Guney et al., 2021). In addition, there is no one superior molecular marker can fulfill all above requirements.

More recently the use of SSR (Simple Sequence Repeat) are widely used in walnut diversity studies. Molecular genetic diversity is measured by studying different molecular genetic parameters such as Na, Ne, I, Ho, He, and other parameters. Different online freely available molecular software's are used for analysis and interpretation of molecular data. High genetic diversity values of novel SSRs identified after screening them on individual seedling genotypes/populations can be further used in more genetic diversity studies of walnut germplasm to obtain more useful information about seedling germplasm collections or populations. The first diversity analysis by the development of SSRs was achieved by Woeste et al. (2002) and Dangl et al. (2005) and generated 30 and 14 SSR primers, respectively, from *Juglans nigra*. After that more studies by different researchers were carried out for the development of polymorphic SSR markers.

In last two years researchers in different parts of the world choice SSR for more reliable characterization of germplasm. For example, Orhan et al. (2020) fingerprinted 34 seed propagated walnut genotypes with 21 SSR loci in Turkey and found total of 135 polymorphic alleles. Shah et al. (2020) reported 23 primer pairs amplified 54 alleles total with 2 to 3 alleles per locus in 84 walnut samples in Jammu and Kashmir. Bujdoso and Cseke (2021) screening 12 SSR markers in Hungarian walnut genotypes and found high diversity. Guney et al. (2021) studied 91 seed propagated walnut genotypes in Turkey by genotyping them with 45 SSR markers. They obtained a total of 390 alleles were amplified with a mean value of 4.06.

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# **CHAPTER 15**

# THE GROWTH PERFORMANCE OF SOME COMMON BEAN VARIETIES RELATED TO DAILY WEATHER TEMPERATURE

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### **1. INTRODUCTION**

There are many of physical environment factors such as temperature, water and nutrients affected the growth and development of crops. Thus, quantifying their effects on crops of these factors is significant to form the basis of simulation models of crop production (Goudriaan et al., 1994). However, the effect of these factors vary noticeably between crop developmental and growth processes. As growth is an increment in common matter resulting from the environmental resources, it is much more affected especially temperature.

Temperature is to cause more to alter in the crop system by influencing the enzymatic activity of crops. Crop development is significantly reliance on high and low temperatures for control of the rate of the development and vegetation stage (Landsberg, 1975). As the rate of crop growth and development varies depending on the temperature the crop exposed to, each species needs specific min, max and optimum temperatures. The increasing temperatures have the capability to remarkably affect crop production (Zhou et al., 2017). Additionally, evaluating crop growth is important due to provide information about climate change, which has become a common problem in society. Actually, it reported that the daily minimum temperatures will increase more quickly than daily maximum temperatures and the increase in the daily mean weather temperatures will be effect undesirable effects on crop yield (Meehl et al., 2007). More specifically, the rising of 2.5°C in the annual mean temperature by 2050 will doubtly decrease common bean productivity (Eitzinger et al., 2018). Hence, understanding the potential impacts of temperature on crop growth and development will provide to develop adaptation strategies to balance these impacts in the future.

The climatic conditions during the growing period of common beans, which are very sensitive in terms of climate demands, are significant for crop growth and development. Common bean (*Phaseolus vulgaris*) is one of the most significant food legumes, and it is the primary source of vegetable protein, minerals, vitamins, dietary fiber and energy especially in less developed countries for human nutrition (Devi, 2021). The total bean cultivated area and production worldwide in 2020 were 35.5 million hectares and 28.84 million tons, respectively. The largest world producers of common beans are India, Brazil, Myanmar, China, Mexico, Uganda and USA, respectively (FAOSTAT, 2021). The most important reason for the low and variable average yield of crops is environmental changes (Newton and Edwards, 2006).

The present study was conducted to analyze the effects of weather temperature (daily max and min temperature) on the growth and development of common bean varieties in different phenological stage.

### 2. MATERIAL AND METHODS

The experiment was carried out from March to July 2021 in the Dicle University, Agriculture Faculty, and Field Crops Department Experiment Farm. The experiment soil is light-alkaline (pH 7.46), clay loam with about 50% clay content. It contains about 0.640% organic matter, 0.032% nitrogen (N), 13.6 kg ha<sup>-1</sup> potassium (K) and 0.188 6 kg ha<sup>-1</sup> available phosphorous (P).

The meteorological data indicated that the total amount of rainfall during crop season was 55.2 mm only in May, with no rainfall in June and July. Mean daily maximum air temperatures ranged from 25.1 in April to 42.6 °C in July. Minimum air temperatures were recorded at 2.2 °C in April and 17.0 °C in July.

Three different common bean varieties (Aras-98, Akman-98 and Noyanbey) were used in the experiment. Seeds of common bean cultivar, Aras 98, which had an indeterminate bush-like growth habit (Type II) were sown on 12th April 2020.

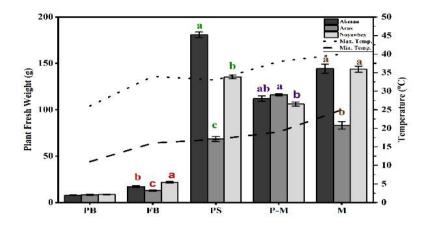
The experiment was arranged in a randomized complete block design with three replications. The plot size was 4 m length, and 5 rows, having row to row distance of 45 cm. The crop was irrigated at field capacity during crop season. The full emergence and full blooming dates were for all plots on 22<sup>nd</sup> April and 21<sup>th</sup> May, respectively. Crop samples were collected at 15 days intervals starting from 20 days after the emerging (DAE) until 95 DAE. The pre-blooming stage (PB), which stage of vegetative mass accumulation, was the stage from 20 days after seedling emergence. The Full-blooming stage (FB) was the time in days between the opening of the first and last flower. The pod setting stage (PS) was defined as the period from the day the pod forming first pod until the day when there was no further rise in pod number. P-M stage is consist from the day the pod setting to maturity. Maturity (M) was the time physiological maturation of crop.

After crops were removed from the plots by hand, were washed off the soil, and dried gently with a soft paper towel to remove free surface moisture. The samples were weighed for fresh weight, and then dried in an oven at 105°C for 24 hours and weighed. An HP Scanjet 3400C was measured leaf area by Winfolia software immediately after sampling. Spectral characteristics of leaves of common bean crops at the flowering stage in different treatment plots were measured by using a GreenSeeker optical sensor. SPAD value was measured by using the SPAD 502 Chlorophyll-Meter at 50% during the pre-blooming and full-blooming stages.

The statistical analysis was performed by JumpPro-13 statistical package program, and graphs were designed by OriginPro-22 excel program. The significance of mean values was analyzed using LSD test (0.05). The correlation coefficient was used for the relationships between traits.

### **3. RESULTS AND DISCUSSION**

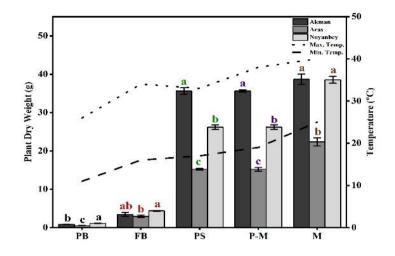
The present study was conducted to analyze the effects of weather temperature on the growth and development of common bean varieties in different phenological stages (PB: Pre-blooming, FB: Full-blooming, PS: Pot setting, P-M: from pod set to maturity, M: Maturity). In research, it was worked on vegetative parts of bean varieties such as plant fresh and dry weight, plant height, leaf area, number of pods per plant, chlorophyll content (SPAD) and green seeker (NDVI) values. The results were given in Figure 1, 2, 3, 4, 5, 6 and 7.



**Figure 1.** Under different growth stages of common bean varieties for crop fresh weight were significant ( $P \le 0.05$ ). The differences among the means denoted by the same letters are not statistically significant. Abbreviations: PB: Pre-blooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

Relationships between crop fresh weight and temperatures at the different development stages were positively correlated with each other (P $\leq$ 0.05). It was determined that young vegetative parts developed and growth started again in two varieties (Akman-98; 180.81 g and Novanbey; 135.29 g) depending on temperature and irrigation in maturity stage. This trait was decrease with interruption of irrigation and yellowing of the lower crop leaves in some varieties at the P-M and maturity stages. Towards the end of the pod setting, if there are enough amount number of pods per plant, the irrigation should be reduced, and to obtain seeds allowed to mature the crop. During this stage, the photosynthetic energy of the crop should be transferred to the pods and seeds instead of vegetative parts, as temperatures above 40 °C were caused flower abortion and should be prevented new flowering. Reichert et al., (2015) reported that common bean growth and development were exposed to adverse environmental factors, especially high weather temperatures, at the full-blooming (38 DAE) and early maturity stage (50 DAE). Additionally, they stated that the average daily weather

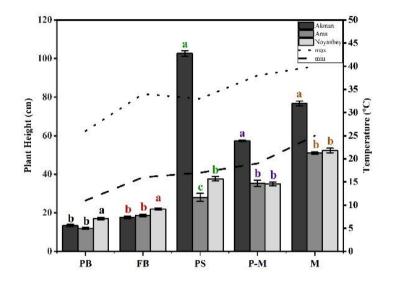
temperature during the growing period was higher than the optimum temperature of 24°C for common beans (Balardin et al., 2000).



**Figure 2.** Under different growth stages of common bean varieties for crop dry weight were significant ( $P \le 0.05$ ). The differences among the means denoted by the same letters are not statistically significant. Abbreviations: PB: Pre-blooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

The effect of rising temperatures on varieties at different growth stages was significant. It was observed that crop dry weight increased as the air temperature increased, but it was not changed at PS and P-M stages. At the maturity stage, crop dry weight increased for all varieties (Akman-98; 38.69 g, Aras-98; 22.34 g; Noyanbey; 38.53 g), due to increased flowers, pods and stem. Actually, the variation of this character was more closely associated with maturity. Additionally, asymmetric max and min weather temperatures had a great impact on crops to high temperatures sensitive such as common bean.

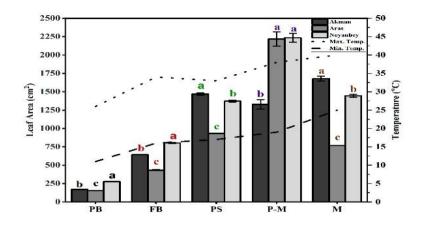
The temperatures varied at different growth stages affected the plant height. The increasing temperatures extended plant height in Aras-98 variety in all development stages. The largest effect of increased temperature was in Akman variety. At the pod setting stage, Akman-98 variety was the tallest (102. 66 cm) compare to other varieties. It was determined that all varieties were similarity in plant height (Akman-98; 17.66 cm; Aras-98; 18.66 cm; Noyanbey; 22.00 cm) at 45 DAE (full blooming stage) (Figure 3).



**Figure 3.** Under different growth stages of common bean varieties for crop height were significant ( $P \le 0.05$ ). The differences among the means denoted by the same letters are not statistically significant. Abbreviations: PB: Pre-blooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

At the PS stage, Akman-98 variety, which had in-determined growth habits, differed from other varieties for plant height. The crop was continue its normal growth and development under optimum temperature and irrigation conditions. The Noyanbey variety was tolerant to low temperatures compared to other varieties, and it. The crop height of this variety decreased during the development period from pod setting to physiological maturity, while it increased in the other growth stages.

It was detected that increasing weather temperature accelerated crop growth and leaf area (Figure 4). This rapid growth will continue as long as normal irrigation conditions were maintained. Therefore, crop growth and development may decline depending on the increase in temperature in waterlimited conditions. For all three varieties, the period between pod setting and maturity was considered to be the maximum period of growth. At the P-M stage, the leaf area increased in two varieties (Aras-98; 31.40 cm<sup>2</sup>, Noyanbey; 31.48 cm<sup>2</sup>) compare to Akman-98 8 (22.94 cm<sup>2</sup>). However, this variety was continued to grow during the maturity stage as it showed indetermined growth habits. The leaf area of other varieties decreased due to leaf wrapping and defoliation from the beginning of the maturity stage.

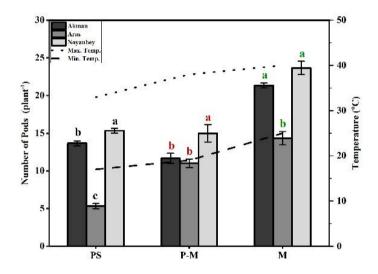


**Figure 4.** Under different growth stages of common bean varieties for leaf area were significant ( $P \le 0.05$ ). The differences among the means denoted by the same letters are not statistically significant. Abbreviations: PB: Pre-blooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

In the stage between pod setting and maturity, while the highest temperature was 40 °C, the lowest was below 15 °C. Actually, all vegetative parts, including leaves, stem and pods, increase in temperatures above 30 °C and under optimum humidity conditions in common beans. Higher temperatures than 30 °C may sometimes reduce flowering and even stop. Common bean growing remains limited in planting areas where the temperature is above 35°C. It is expected that physiological maturity completed in places where the average temperature is 30 in April, May and June. However, the temperature, which does not adversely affect flowering, can increase yield including vegetative parts in high temperature areas with high humidity. In this study was determined that vegetative parts such as leaf

area and plant height increased at suitable temperatures. Barbano et al., (2001) reported that the remaining temperature above 30°C during the day negative effects on common bean growth. In some research, it was detected that crop leaf area and crop height were reduced due to increased air temperature as a result of tillage (Silva et al., 2006) and limited availability of water (Gubiani et al. 2014).

The number of pods per plant increased in 30 °C weather temperature at maturity stage for all varieties. While the disruption in irrigation in the P-M stage negatively affected the pod setting of Akman-98 and Noyanbey varieties, Aras-98 variety was not affected (Figure 5).

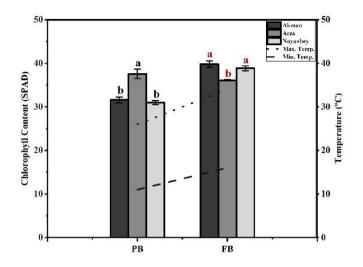


**Figure 5.** Under different growth stages of common bean varieties for number of pods were significant ( $P \le 0.05$ ). The differences among the means denoted by the same letters are not statistically significant. Abbreviations: PB: Pre-blooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

Common bean was more affected by high temperature during the reproduction development stage including the pre-blooming, full blooming and pod setting stages (Shonnard and Gepts 1994; Balardin et al. 2000). Gross and Kigel (1994) stated that temperature increases during the stage of

reproductive development caused stress in crops, and concluded that low pollen viability and impaired female performance in a large proportion of the flowers. They revealed that this case had a negative effect on pod and seed setting. As high temperature effects flowers and pods, seeds, and seed mass (Hoffmann Júnior et al., 2007), temperatures above 30°C during the day provide adversely effects on growth and development of crop (Massignam et al. 1998). Actually, Rainey and Griffiths (2005) reported that 30 °C temperature increased the number of pods in the generative period. Additionally, Massignam et al., (1998) reported that the max temperatures above 28°C related to water deficit at blooming period may cause yield reduction in common beans. Konsens et al., (1991) determined that raising night weather temperature from 17°C to 27°C extremely decreased pod production, while increase during day weather temperature from 22°C to 32°C had low effects. In addition, they stated that the pre-blooming and pod setting stages were the plant stages most sensitive to night temperature.

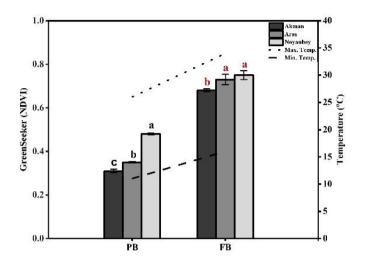
In our study, low soil wet content and high weather temperature during the P-M stage possibly affected reproductive organs. Therefore, the irrigation may be performed to decrease effect of uncontrollable environmental factors including high weather temperature and water deficit on crop growth and development.



**Figure 6.** Under different growth stages of common bean varieties for chlorophyll content were significant ( $P \le 0.05$ ). The differences among the means denoted by the same letters are not statistically significant. Abbreviations: PB: Pre-blooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

It seems that the chlorophyll content (SPAD) and NDVI values, which gave information about plant health, was not adversely affected related to the increase in temperature (above 35 °C) due to normal irrigation conditions. While the chlorophyll content of Aras-98 variety (in preblooming stage; 38.56 and full blooming stage; 36.10) was higher in the preblooming stage compared to other varieties, the opposite case was observed during the full blooming stage (Figure 6). Sage and Sharkey (1987) reported that photosynthesis rapidly increased related to increasing weather temperature. The other researchers detected that the increased minimum temperatures exhibit a larger impact on grain yield than on vegetative growth (Hatfield and Prueger, 2015).

For most crop, vegetative development stage generally has a higher optimum temperature than for generative development. The vegetative development increases as temperatures rise to the crops optimum level (Figure 7).



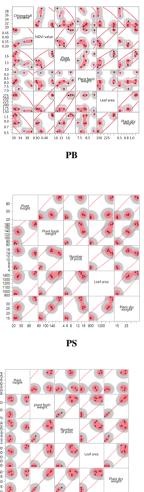
**Figure 7.** Under different growth stages of common bean varieties for greenseeker (NDVI) were significant ( $P \le 0.05$ ). The differences among the means denoted by the same letters are not statistically significant. Abbreviations: PB: Pre-blooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

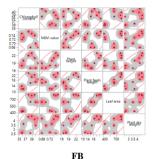
The green seeker (NDVI) value increased for all varieties in both the pre-blooming (Akman-98; 0.31, Aras-98; 0.35, Noyanbey; 0.48) and full blooming stage (Akman-98; 0.68, Aras-98; 0.75, Noyanbey; 0.78). While the Noyanbey variety was in the forefront for this value in the pre-blooming stage, the Aras-98 and the Noyanbey varieties were in the forefront during the full blooming stage (Figure 7).

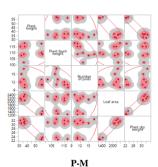
The results obtained in the correlation analysis carried out to determine the relationship between the characteristics examined in different developmental periods in the experiment were given in Figure 8.

During pre-blooming stage (PB), the plant height was positively correlated with the green seeker value. The leaf area was positively correlated with the green seeker value and plant height. Plant dry weight was negatively correlated with chlorophyll content, however positively correlated with green seeker value, plant height, plant fresh weight and leaf area ( $P \le 0.01$ ) (Figure 8).

In full blooming stage (FB), the plant height was positively correlated with the green seeker value (P $\leq$  0.05). The leaf area was positively correlated with plant fresh weight ( $P \le 0.01$ ). The plant dry weight was positively correlated with plant height was positively correlated with plant fresh weight (P $\leq$  0.01), and plant fresh weight and leaf area was positively correlated with plant fresh weight ( $P \le 0.05$ ) (Figure 8).







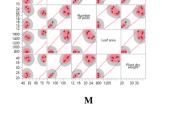


Figure 8. Simple correlation coefficients between traits of common bean varieties evaluated air temperature conditions at Diyarbakir for all development stages. Abbreviations: PB: Preblooming stage, FB: Full-blooming, PS: Pod setting, P-M: From pod setting to maturity, M: Maturity.

In pod setting stage (PS), the plant fresh weight was positively correlated with plant height (P $\leq$  0.01). The number of pods was positively correlated with plant fresh weight, and leaf area correlated with plant height (P $\leq$ 0.05), plant fresh weight and number of pods (P $\leq$  0.01). However, plant dry weight was positively correlated with all traits.

From pod setting to maturity stage (P-M), the leaf area and plant dry weight was negatively correlated with plant height, and plant dry weight positively correlated with leaf area ( $P \le 0.01$ ).

In maturity stage (M), the number of pods was positively correlated with plant fresh weight (P $\leq$  0.01). The leaf area was positively correlated with plant height (P $\leq$ 0.05), plant fresh weight and number of pods (P $\leq$ 0.01). The plant dry weight was positively correlated with all traits except for plant height (P $\leq$ 0.01).

Some researcher reported that positive significant correlations were observed on changed temperatures in crop dry weight, pod setting, pod weight and yield among bean genotypes (Paul et al., (1991). However, Kulig et al., (2011) revealed that between number of seed and seed weight with average monthly temperature in April was highly correlation.

#### CONCLUSION

In this study, the effect of temperature on beans under normal irrigation conditions at different plant development periods was investigated. The response of plant parts including plant height, plant fresh and dry weight, leaf area and chlorophyll values to temperatures during all development periods from pre-bloom to maturation period were investigated. It was been determined that the rising air temperature without creating heat stress had a positive effect on plant growth. Also, high temperature increased the vegetative part of the plant more than the generative part. In areas exposed to high temperatures, it was necessary to apply sufficient amount of irrigation water to increase the number of pods and seeds per plant.

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