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Preface

Walnut is one of the most important fruits which has been defined as a "Super food" in recent years that appreciated by many for its taste and aroma and evaluated in the Nuts category. This food is consumed with love, is a special fruit with a nutritional and consumer-friendly dimension that provides physiological benefits for human health. Consumers are willing to pay good money for these features that help ensure strong growth in worldwide production and contribute to the country's economy. Walnut production in both nuts and other products has increased significantly in the last two decades in traditionally leading walnut producing countries such as China, USA, Chile, Turkey and Ukraine. Also during this time, China and the USA are the largest producers in the world, and production in China is also increasing dramatically with the rise of the middle class in the world's most populous country. The relatively long juvenile period and many labor-intensive processes take place in walnut cultivation, from pollen collection, hand pollination and fruit protection from birds/insects/diseases to manual harvesting. However, as a result of rapid developments in the field of molecular genetics in recent years, it shows great promise for wider improved diversity characteristics and more efficient studies in walnut breeding.

I think it is of greater importance among future walnut growers, who will have to adopt more complex, site-specific production strategies in terms of plant materials and cultural practices. Considering global warming in recent years, taking into account current and future climatic conditions, potential genetic advances (such as proprietary varieties resistant to biotic and abiotic stress factors), may cause limitations in the application of certain modern orchard technologies for walnut producers. Therefore, these issues need to be addressed. For this purpose, characterization and conservation of the diversity of walnut genetic resources and broad and multidisciplinary action plans are needed for this purpose.

For this reason, in recent years, there are brochures, books, etc., about the book on walnuts, which he has included in his new studies, and the human health effects of walnuts. There were not many documents and it was stated that both students and those who were interested in walnut needed a new book about walnuts.

We need vitamins and minerals for our health. These nutrients occur naturally in plants. However, our busy lives make it difficult to maintain a balanced diet on a regular basis. Our daily activities often prevent us from consuming enough of grains, fruits, vegetables and other products. Daily multivitamin and mineral support may not provide us with all the nutrients we need. In addition, we cannot get enough antioxidant substances that the body needs on a daily basis. For this, some of the necessary nutrients can be supplemented with many plants that are functional food sources and nutritional supplements obtained from these plants.

I thought this was mainly due to the fact that new books were not written. For this reason, I wanted to bring to the agenda the bioactive compounds it contains and walnuts, which have become increasingly popular as a support product for human health, which has been very important to both the country's economy and the producer economy and the last years. While the book was being prepared, it was prepared by scanning current sources together with our expert academician friends.

In the book; will help students, research scientists and individuals who are curious about walnuts to better know the walnut, which is undoubtedly the most interesting area of human health in recent years, to understand more, to research more deeply and finally to benefit more from them. It has been prepared in the light of scientific findings and academic rules in order to help you. Almost every subject related to walnut is also explained in detail with charts, graphics, pictures and figures. The book provides readers with some innovations in terms of topics and approach. With this compiled book, it is aimed to inform the producers about walnut cultivation, which is currently grown in our country and which is in demand every day, and I hope it will be useful to all our readers.

I would like to thank all my friends and IKSAD International Publishing House publication staff who contributed to the preparation of the walnut book. I hope that this printed book study called "Super Food" in Walnut will be useful to all academicians, producers and students, as well as those who are interested in walnuts.

December 2021

Editor

Assoc. Prof. Muhammet Ali GUNDESLİ

Acknowledgements

This publication helps connect scientific research initiatives and enables scientists to share their ideas with colleagues and enhance their research, careers and innovation.

Part I

Walnut Production, Trade and Forecast

Dr. Serhan Candemir[1]

Walnuts are grown all over the world, from the Carpathians to Turkey, Iran, Iraq, Afghanistan, Southern Russia, India, and Korea (Sen et al, 2006). Although it is documented in many scientific publications that people in Anatolia have known this plant for at least 3000 years and have benefited from various products of this plant, walnut cultivation is carried out at an altitude of 1000-2000 m in the Swiss Alps and an altitude of 1730 m in Turkey's Munzur Mountains (Ferhatolu, 2001; Kilci, 2015).

The fossils obtained from cultivation areas are the most important evidence that this plant has been grown in wide geography for centuries due to the walnut tree's high adaptability to environmental conditions. According to some sources, the walnut dates back to BC. They claim that it took 750-500 years to travel from Iran to Greece. According to the sources cited above, the history of walnut cultivation

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dates back to ancient times (Akarçay, 2007). Turkey, which has a long and deep-rooted fruit-growing culture, is the home of walnuts and many other fruit species (Koyuncu and Aşkın, 1999).

Preferring fruit varieties that can be adapted to various climate and soil conditions around the world is important because it creates alternatives that can serve a variety of functions (Gülcan et al 2000). The fruits produced not only supply raw materials to many sectors, but also meet the food needs of people. The production and consumption of hard-shelled fruits, which contain valuable components such as protein, fat, and carbohydrates for a healthy diet, is increasing daily. Walnut is a popular species for hard-shell fruit cultivation.

With the impact of epidemics all over the world, healthy nutrition has become an important issue. Hard-shelled fruits, a fast-consuming food product, are among the producers' investment preferences in terms of aquaculture, economy, and nutrition. Walnut is regarded as an important agricultural product throughout the world due to its wide range of applications, with its fruit, leaves, timber, and green shell all being economically valuable. The fact that it is a raw material in many industrial sectors such as food, furniture, and medicine has allowed for an increase in demand for walnuts both globally and Turkey.

Walnut cultivation in Turkey is mostly done for domestic consumption and domestic consumption. The walnuts produced cannot meet the consumption, especially in recent years, walnuts are imported at an increasing rate, but although it changes according to the years, very little of it is exported to different countries as shelled and kernel walnuts. While Turkey's walnut proficiency index was approximately 94% in 2000, it was 73% in 2019 (Turkstat, 2021).

The majority of walnut cultivation in Turkey is done for domestic and household consumption. Walnuts are being imported at an increasing rate, but very little of it is being exported to various countries as shelled and kernel walnuts, even though production cannot keep up with demand, particularly in recent years. While Turkey's walnut proficiency index was around 94% in 2000, it is now only around 76% in 2019 (Turkstat, 2021).

Walnuts in the World

In many countries around the world, 4.5 million tons of production is made on a total area of 1.3 million hectares. While there were no major changes in the walnut planted areas between 1965 and 1985, it was determined that the walnut planted areas increased rapidly every year as of 1986. Between 1965 and 1985, walnut planted areas, which changed between 160,000 ha and 175,000 ha, reached 600,000 ha in the early 2000s, 1 million ha in 2010 and 1.3 million today (FAO, 2021).

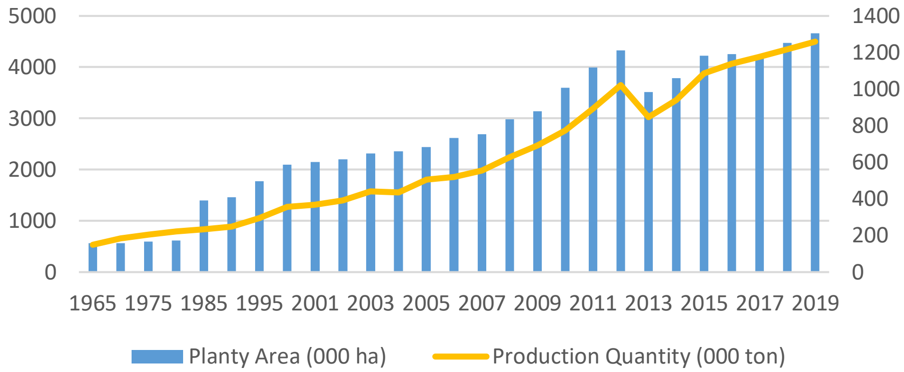


Figure 1. World Walnut Planting Area and Change in Production Quantity

Walnut production is the subject of statistics in 59 countries around the world, and the world walnut production is around 4.5 million tons. China produces about half of this production with a production of 2.5 million tons. China is followed by the USA with 0.6 million tons of production, Iran with 0.3 million tons and Turkey with 0.2 million tons of production. With these production values, 81% of the world walnut production is realized by these four countries (FAOSTAT, 2021).

When the walnut production amount is analyzed by countries, according to 2019 data, the largest planting area in the world is 630,000 ha, 48% of which belongs to China. China is followed by USA with 11%, Turkey with 9% and Iran with 3%. Turkey, which is 3rd in the world in terms of planting area, ranks 4th in terms of production amount due to differences arising from productivity. 56% of the world walnut production is carried out by China, 13% by USA, 7% by Iran and 5% by Turkey. Yield is one of the most important factors affecting the amount of production. When the yield data by countries were examined, it was determined that the lowest yield was found in Mexico with 1.68 tons/ha, while the highest yield was found in Uzbekistan with 10.43 tons/ha. Factors such as the preferred variety, production system and climate can change the yield (FAOSTAT, 2021).

Table 1. Production, cultivation area and yield based on countries

	Production Quantity (ton)	Cultivation Area (ha)	Yield (ton/ha)
China	2521504	631330	3,99
USA	592390	147710	4,01
Iran	321074	44780	7,17
Turkey	225000	124553	1,81
Mexico	171368	102068	1,68
Ukraine	125850	13900	9,05
Chile	122950	40801	3,01
Uzbekistan	50660	4857	10,43
Other Countries	367646	195350	
World	4498442	1305349	3,45

Source: Faostat, 2021

World Foreign Trade

In the world's foreign trade, walnuts are traded in two different ways as shelled walnuts and unshelled walnuts. While the world's (shelled + unshelled) walnut import was around 154 thousand tons in 2000, it increased to 711 thousand tons in 2019. World walnut (shelled + unshelled) exports, on the other hand, were 180 thousand tons in 2000 and increased to 850 thousand tons in 2019. It has been determined that the share of the walnut market in the world foreign trade shows an increasing trend.

Table 2. World walnut foreign trade by years

	Import Quantity (ton)			Export Quantity (ton)		
	Without Shell	Shelled	Total	Shelled	Without Shell	Total
2000	88029	65880	153909	72567	106565	179132
2005	91234	115378	206612	137678	129018	266696
2010	198973	150329	349302	191379	214325	405704
2015	189968	205836	395804	267123	282831	549954
2016	238162	218002	456164	271162	364204	635366
2017	251410	246882	498292	295886	320008	615894
2018	281442	243860	525302	294194	335226	629420
2019	434217	277403	711620	344049	503730	847779

Source: FAOSTAT, 2021

According to the data of 2019, Germany is the largest walnut importer country in the world with approximately 42.000 tons, followed by Japan, Turkey and Spain. When the shelled walnut export data is analyzed, USA comes to the forefront as the most important walnut exporting country with 122 thousand tons, followed by Mexico, Ukraine and Chile.

Table 3. World walnut in shell foreign trade by countries

Shelled Import (ton)			Shelled Export (ton)		
	Import Quantity	Share (%)		Export Quantity	Share (%)
Germany	42425	15,3	USA	121980	35,5
Japan	17412	6,3	Mexico	54899	16,0
Turkey	16978	6,1	Ukraine	36829	10,7
Spain	16702	6,0	Chile	31646	9,2
Korea	12539	4,5	China	20524	6,0
France	11972	4,3	Moldova	15836	4,6
Netherlands	11712	4,2	Germany	13632	4,0
Canada	11637	4,2	Turkey	6200	1,8
Other Countries	135990	49,0	Other Countries	42503	12,4
World	277367	100,0	World	344049	100,0

Source: FAOSTAT, 2021

Turkey ranks first in the import of unshelled walnuts with 88 thousand, while United Arab Emirates ranks second with 55 thousand (12.6%). Together with Italy, Iran and Kyrgyzstan, 5 countries realize approximately 60% of the world's unshelled walnut imports. When the export of unshelled walnuts is analyzed, USA stands out as the largest exporting country with 157 thousand tons. USA is followed by China, Chile and United Arab Emirates. 4 countries realize 60% of the world's shellless walnut exports.

Shelled walnut producer prices by country are examined in table 5. According to 2019 data, while the average world walnut price is $3.12, it is $3.55 in Turkey. The highest walnut price is in Palestine with $10.2 and the lowest price is in Peru with $0.32. Prices in the USA, which is the largest walnut exporter in shell, were at the level of $2.2, below the average world walnut prices. This situation provides the USA with competitive power in world trade due to its price advantage.

Table 4. World without shell walnut foreign trade by countries

Without Shell Import (ton)			Without Shell Export (ton)		
	Import Quantity	Share (%)		Export Quantity	Share (%)
Turkey	88053	20,3	USA	157554	31,3
United Arab Emirates	54797	12,6	China	74193	14,7
Italy	45893	10,6	Chile	73792	14,6
Iran	41436	9,5	United Arab Emirates	49487	9,8
Kyrgyzstan	36956	8,5	Mexico	47800	9,5
Mexico	35904	8,3	France	25396	5,0

Spain	15331	3,5	Ukraine	20027	4,0
India	14973	3,4	Turkey	16587	3,3
Iraq	12606	2,9	Argentina	6445	1,3
Morocco	10840	2,5	Kyrgyzstan	4434	0,9
Germany	10504	2,4	Australia	3266	0,6
Other Countries	66923	15,4	Other Countries	24749	4,9
World	434216	100,0	World	503730	100,0

Table 5. Shelled walnut producer prices by country

Countries	Price (USD/kg)
Palestine	10,2
China	7,5
Iran	4,8
Germany	4,5
Mexico	3,8
Turkey	3,6
USA	2,2
Azerbaijan	1,5
Peru	0,4
World	3,12

Walnut Production in Turkey

Turkey is an important country that ranks 4th in terms of world walnut production. The amount of walnut production by years is examined in chart 2. It is observed that the amount of production has been on an increasing trend since the beginning of the 2000s, and there has been an increase in the amount of consumption with the increase in the amount of production. Both as a requirement of a healthy life and with the effect of population growth, the rate of increase in the amount of consumption after 2014 was above the rate of increase in the amount of production. This situation caused a decrease in the proficiency level of walnuts in Turkey. Walnut proficiency level, which was around 90% at the beginning of the 2000s, declined sharply to 70% after 2014.

While trying to fill the supply gap with imports, support policy tools are used by the Ministry of Agriculture and Forestry for the establishment of new walnut orchards. For the establishment of walnut orchards, the Ministry of Agriculture and Forestry provides input support, organic agriculture support, good agriculture support and certified sapling use supports. In addition to these supports, 25% of the project investment value is given as a grant in walnut orchard facilities within the scope of private afforestation. In addition, low interest loans applied in agricultural production reduce interest rates up to 95% in walnut production (TOB, 2021).

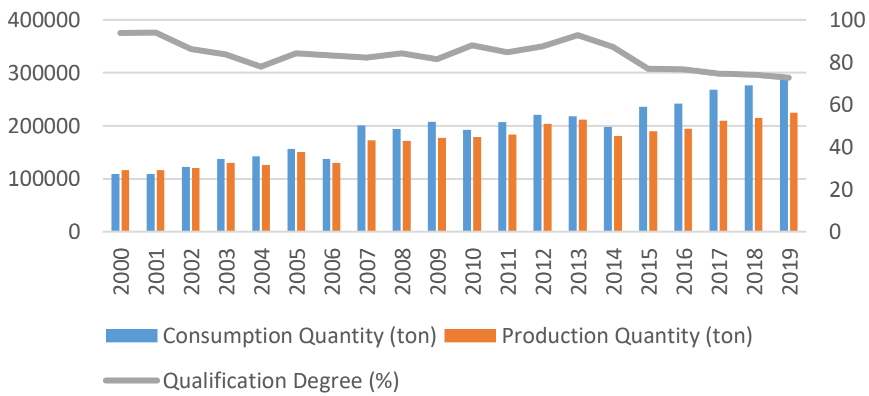


Figure 2. Turkey Walnut Production, Consumption and Sufficiency Degree

According to 2019 data, Turkey produced around 225 thousand tons of shelled walnuts. Hakkari with 11.5 thousand tons and Kahramanmaraş with 11.4 tons are among the most important provinces in walnut production in Turkey. The provinces of Hakkari and Kahramanmaraş, Mersin, Bursa and Denizli follow it. While Denizli stands out as the walnut planting area in Turkey, the provinces of Manisa and Bursa follow Denizli. It is expected that the leading provinces will change for production, as the production amount of Turkey increases as the trees that do not bear fruit reach the yield age.

Table 6. Walnut production by provinces in Turkey

	Production Quantity (ton)	Number of fruit bearing trees (Number)	Number of non-fruiting trees (pieces)	Planting area (ha)
Hakkâri	11682	501500	212000	2725
Kahramanmaraş	11436	494290	417831	6587,5
Mersin	10838	306389	183076	3161,8
Bursa	10837	372023	573938	7270,2
Denizli	8941	563218	708511	8073
Çorum	8581	605444	301920	3602,1
Sakarya	5830	240765	172725	2691,3
Antalya	5825	263605	103766	1928,7
Manisa	5394	351106	924314	7712,2
İzmir	5305	208297	95613	1468,9
Other provinces	140331	7343889	6310623	79332
Türkiye	225000	11250526	10004317	124552,7

Source: Turkstat, 2021

Forecast in Turkish Walnut Production

In this part of the study, the time series of production, consumption, export and import amounts of walnut production between 2000-2019 were examined. 4-year estimations were made with time series and double exponential correction method.

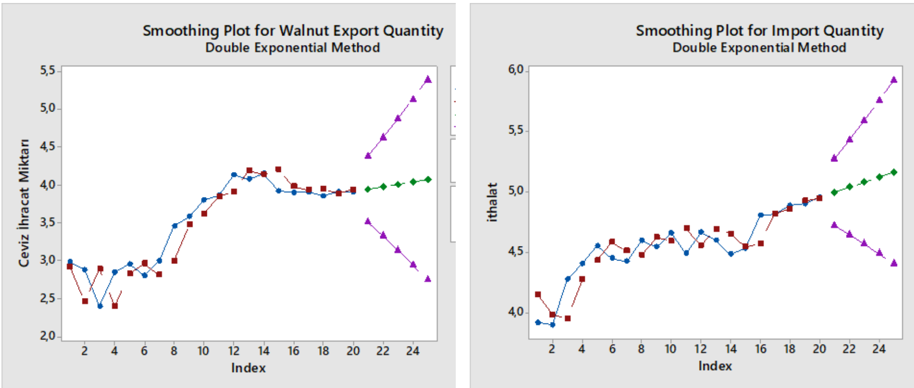
Table 7. Self-sufficiency indexes and calculations in Turkish walnut production

Parameters	Formulation	2019 values
Production Quantity (ton)		225000
Consumption Quantity (ton)		294698
Import Quantity (ton)		90525
Export Quantity (ton)		8180
Self-Sufficiency Ratio (SFL)	SFL = Production / Consumption	%76,3
Import Dependency Index (IDI)	IDI = Import/Consumption	%30,7
Exportability Index (EI)	EI = Export /Consumption	%2,8

According to the estimation results, Turkish walnut proficiency indices were calculated. The formulations of the proficiency indices and their 2019 actual values are given in table 7.

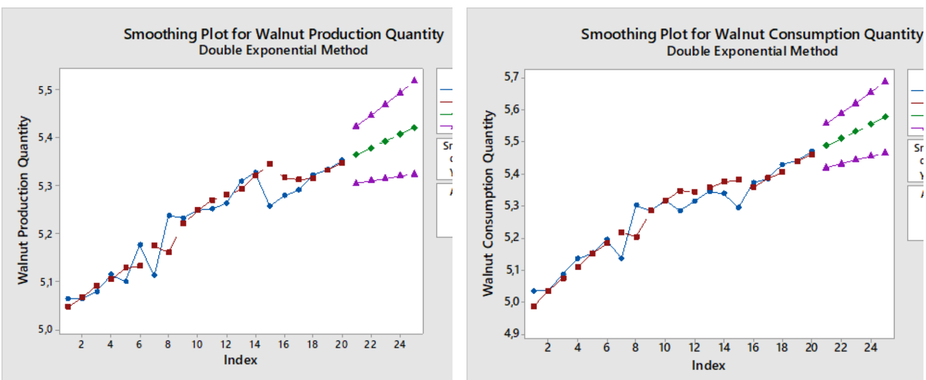
Walnut Production, Consumption and Foreign Trade Forecast

The normality test for the time series of 2000-2019 was applied (Kolmogorow-Smirnow) and it was determined that the data set had a normal distribution. 3-year (2020-2022) forecasts for walnut production, consumption, exports and imports were made using the double exponential recovery method.



a) Forecast Export Quantity b) Forecast Import Quantity

Figure 3. Forecast of walnut foreign trade amount between 2020-2022



a) Forecast Production Quantity b) Forecast Consumption Quantity

Figure 4. Forecast of walnut production and consumption trade amount between 2020-2022

Turkey walnut production, consumption, export and import quantity have been estimated separately and given in table 8. The data for 2019 shows the realized values. While the walnut production amount in Turkey was 225 thousand tons in 2019, it is expected to increase to approximately 240 thousand tons in 2022 with an increasing trend in the forecast years. Similarly, it is estimated that the amount of consumption will increase and reach the level of approximately 320 thousand tons. It is predicted that the export amount, which is 8 thousand tons, will reach the level of 10 tons in 2022, and the import quantity will reach the level of 120 tons.

Table 8. Forecast of aquaculture production and foreign trade between 2020-2022

	Production Quantity (ton)	Consumption Quantity (ton)	Export Quantity (ton)	Import Quantity (ton)
*2019**	*225000*	*294698*	*8180*	*90525*
2020	227517	297593	8819	99405
2021	233143	308032	9474	109441
2022	238770	318471	10177	120490

** realized value*

Forecast of Qualification Indices

Self-Sufficiency Ratio (SCR) and Import Dependency Indices (IDI) are used to measure the extent of countries' self-sufficiency in any product. Both indices measure how much of a country's total supply is met by domestic production or by imports. In addition to the two ratios, the Exportability Index is used to show how much of the production is exported (Candemir and Dagtekin, 2020). Examining the adequacy indices related to walnut production will enable to comment on the future of the sector.

Table 9. Forecast of proficiency indices for 2020-2022

	Self-Sufficiency Ratio (%)	Import Dependency Index (%)	Exportability Index (%)
*2019**	*76,3*	*30,7*	*2,8*
2020	76,5	33,4	3,0
2021	75,7	35,5	3,1
2022	75,0	37,8	3,2

** realized value*

In Table 9, proficiency indices related to Turkish walnut data were calculated. In 2019, the self-sufficiency rate of walnut production was 76.3%. It is estimated that walnut production self-sufficiency rate will decrease to 75% in 2022. In addition, it is estimated that the import dependency ratio, which is 30%, will increase to 37.8% in 2022 and the exportability ratio will be 3.2% with a small increase.

GENERAL EVALUATION

Turkey is a country with a high walnut production potential. It is estimated that the demand for walnuts all over the world has increased over the years and will increase in the coming years. Walnut, which is a healthy fast consumption product, seems likely to increase for consumption due to the increase in the world population and the effect of epidemics.

The fact that Turkey walnut self-sufficiency rate will decrease to 75% in 2022 will increase Turkey's import dependency ratio. For this reason, it is very important for Turkey to increase its walnut production areas in order to gain a place in the world walnut market and become a self-sufficient country.

Walnut production in private afforestation areas should be an obligatory production activity, and it is necessary to turn the products produced into processed products with high benefit. The regions where walnut cultivation is carried out are generally disadvantaged regions where there are sloping lands, where the altitude is high, and one-year agricultural production cannot be done. An important way to ensure regional development is to carry out studies that increase the welfare level of producers in their own regions. In this respect, it is thought that by increasing the organization in walnut production regions, the realization of processing facilities through organized structures will contribute to regional development.

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Part II

Flowers and Fertilization Biology in Walnuts

Assoc. Prof. Hakan KELES[1]

In fruit species, the presence and shape of flowers on the tree are defined in 3 different ways. Hermaphrodite is the condition in which stamens and pistils are present on the same flower and the same plant. The presence of anthers and pistils on the same plant but in different places is called monoic. The presence of stamens and pistils both on different plants and in different places is called dioecious (Figure 1) (Bilir and Ulusan, 2010).

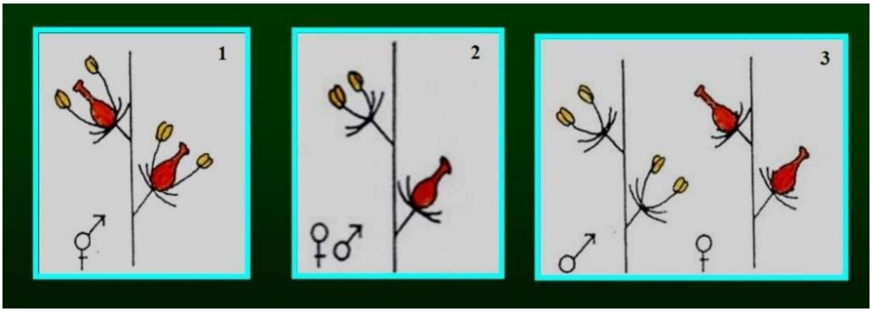

Figure 1. 1: Hermaphrodite flower structure: Male and female flowers together and on the same plant, 2: Monoic: Male and female flowers in separate places and on the

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same plant, 3: Male and female flowers in separate places and on different plants (Anonymous, 2021a)

Walnut (*Juglans regia* L.) trees are among monoecious plants in terms of flower biology (Sharma et. al., 2016; Eser et. al., 2019; Kafkas et al., 2020; Okatan et al., 2021). This word, called monoic, is actually of Greek origin and is formed from the combination of the words "Monos" meaning "single" and "Oikos" meaning "house" expressing that the male and female flower lives in the same house (Schmidt and Joker, 2001; Mueller et. al., 2015). This means that male and female flowers are found separately on the same tree, but in different places (Polito, 1998).

The presence of male and female flowers in different places on the same tree does not require that these flowers bloom at the same time. In this regard, in *J. regia*, male and female flowers become active at different times according to a certain order and sequence. This situation is called dichogamy (Akça and Şen, 1995; Shu-Ganga et. al., 2011, Guney et. al., 2021).

In monoic structure, male and female flowers are not found in the same structure and flowers can occur on shoots of different ages. The flowers that occur at the ends of the annual shoots of the same year are called female flowers, and the catkins formed on the shoots of the previous year are called male flowers (Figure 2).

Figure 2. (A) Female flowers (Photo by: Carlos Coutinho, Anonymous, 2021b), (B) Male flowers (Photo by: H. Zell, Anonymous, 2021c)

1. Female Flowers

Female flowers are usually formed at the ends of the shoots and 1-3 are found together. However, cases, where 10-18 female flowers coexist at the ends of the shoots in walnut trees, have also been encountered. These trees can be used as parents in studies with the aim of productive variety breeding. As in all fruit varieties, the formation and time of female flowers in walnut trees cultivars according to the genetic structures of the cultivars. Although this timing sometimes seems to change due to ecological differences, it usually shows a stable attitude. In a study on the inheritance of different characters in walnuts, it was reported that the heritability of characters such as leafing date, female flower activation date, first pollen dispersal date was high (Janick and Moore, 1979; Akça, 2009). This shows that ecology has little effect on these characters. (Table 1).

Table 1. Heritability of some important characters in walnuts (Janick et al., 1979)

Characters	**Heritability**
Leafing date	0,96
Receptive time of female flowers	0,93
Pollen distribution	0,91
Harvest date	0,85
Fruit height	0,82
Number of quality walnut kernel	0,08
Yield	0,07

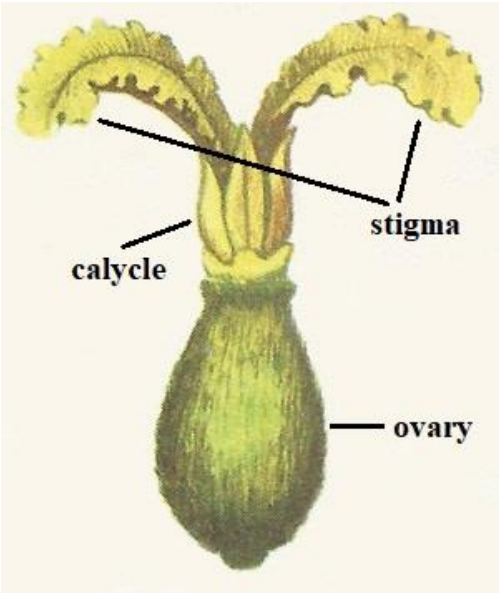

Figure 3. Female Flower (Anonymous, 2021d)

There are no petals in female flowers in walnuts. Although there are 3-6 sepals, the four-part cover leaves (perianth) and bracticle leaves of the flowers are fused with the ovary. (Şen, 2011). The ovary of female flowers is inferior and has two carpels. The stigma, where the pollen is attached, is large lobed and has a recessed structure for the pollen to hold more easily (Polito, 1998; Akça, 2009; Jankovic et al., 2021). Female flowers begin to develop in April. Towards the period when the stigma accepts pollen (receptive period), the stigmas turn from yellow to a darker orange color and the stigmas are opened (approximately 45°). In the receptive period, the stigmas are covered with a sticky liquid to better capture the pollen. This period, when the embryos of female flowers are fertile, can be detected by the presence of sticky fluid on the stigmas (Şekil 4). Under optimum ecological conditions, stigmas of the female flowers are receptive for up to seven days. At the end of the flowering period, which lasts for an average of 20 days, the stigmas have completed their tasks and then dry up (Krueger, 2000; Akça, 2009).

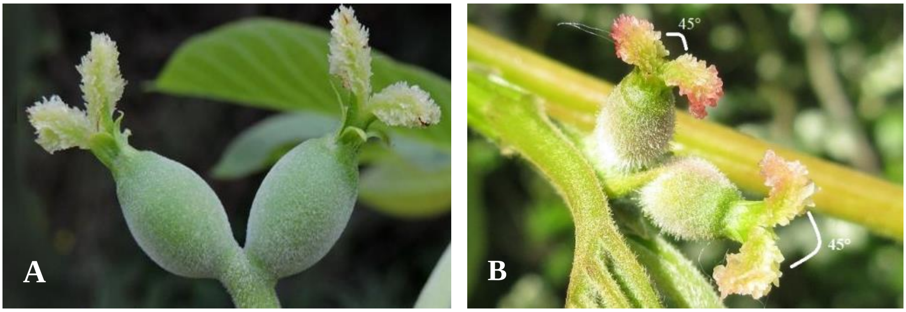

Figure 4. (A) Non-receptive Female Flower (Photo by: Anil Thakur, Anonymous, 2021e), (B) Receptive Female Flower (Anonymous, 2021f)

2. Male Flowers

On the contrary female flowers formed on annual shoots, male flowers are located on structures called catkins formed on the shoots of the previous period (Latorse, 1985). Catkins, which contain male flowers that distribute pollen, are first miniature cone-shaped, conical and gray on the branches, and they begin to develop with the spring period (Krueger, 2000). The catkins, which are 2-3 mm in length when they first form, reach 10-15 cm in length when they complete their development, and the maturing catkins hang down. An axis passes through the center of the catkins and the male flowers are attached to this axis by a stem. Brackets and perianth leaves of male flowers together formed a six-part cover structure (Şen, 2011) (Figure 5).

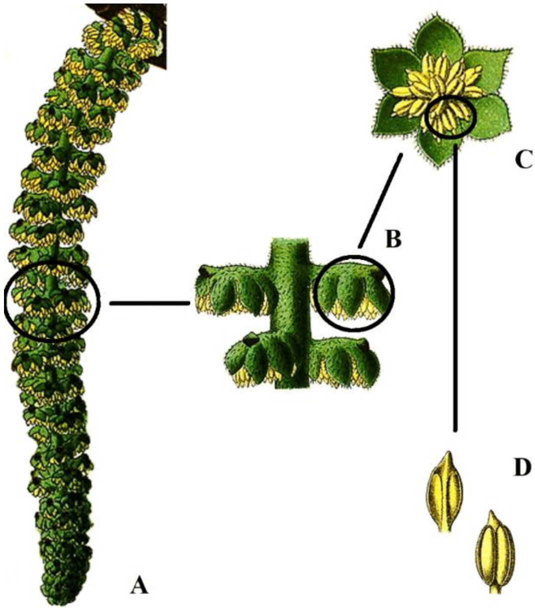

Figure 5. A: Catkin, B: Male flowers attached to the catkin axis, C: Bottom view of male flower, D: Anthers (Modified from, Köhler, 1887)

Each male flower has an average of 12-18 stamen. When all flowers are considered in a catkin, there are 150 male flowers, and about 2 million pollen is produced from a catkin that is ready for distribution. Catkin buds that are formed in the previous development period on the shoots are differentiated with the development period of the new year. It is known that a ripe walnut tree can produce 5-20 billion pollen from an average of 5000 catkins. 10-25% of the produced pollen can survive and pollinate female flowers (Wood, 1934). A catkin that has reached the end of pollen distribution loses its vitality, turns black, and finally falls off.

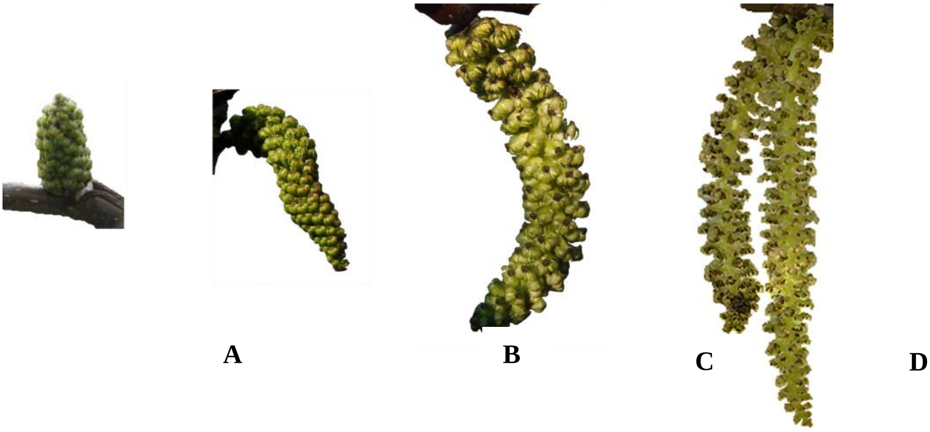

Figure 6. Male flower development stages

3. Fertilization Biology

Walnut (*J. regia*) flowers do not have attractive petals and tempting nectar extracts like insect-pollinated (anemophilous) plants. For this reason, pollination between walnut trees is provided by wind and walnut trees produce a lot of pollen to avoid pollination problems.

There is no evidence of infertility or incompatibility problems that can be encountered in different fruit types in walnut trees that do not have problems in fertilizing each other. Although all walnut trees can fertilize each other, the most important issue in fertilization is the periods of receptive when male and female flowers are active. Because of this situation, walnut plants are classified in the group of dichogamy plants.

3.1. What is dichogamy?

In walnuts, the pollen distribution time of male flowers and the period in which female flowers accept pollen are at different times (Forde and Griggs, 1975). This situation is called dichogamy which term was first used by Sprengel in 1793 (Stout, 1928).

3.2. Factors affecting dichogamy

Dichogamy tendencies of cultivars are fixed by genetic traits, but some factors can push or reverse flowering times. These changes are very limited. The most important factors affecting the flowering times are the age of the plants and environmental factors (Batu et al., 2009). It is very important to know which cultivars to choose in order to ensure pollination in the walnut orchard and to know which factors will affect the flowering behavior and how. Factors affecting flowering times are mentioned below:

- The age of plants is one of the important features that affect the dichogamy. Young walnut trees have a greater tendency to dichogamy and, on these trees, male flowers mature earlier than female flowers (protandrous) (Wood, 1934; Akça, 2009).

- Warm weather is another important factor affecting the dichogamy. In particular, staminate flowers react more quickly to warm weather than pistillate flowers (Wood, 1934; Cosmulescu et. al., 2010).
    - ❖ In a warm season following a cold winter, all parts of the plant tendency to grow rapidly. However, this is more valid for male flowers compared to female flowers. In such cases, male flowers develop much more rapidly, and in protandry cultivars, the flowering of male flowers ends long before the female flowers become receptive, and dichogamy is certainly occur.
    - ❖ In warm spring times accelerate catkins development, but female flowers are not affected this condition much. Under these conditions, protogenetic cultivars may behave like homogamy, especially in cultivars where the flowering interval between the two flower types is less than 3-4 days.
    - ❖ Cool weather after a warm winter can cause the opposite situation in flower behavior. In such cases, the protogenic feature is emphasized. The effects of weather and climate can be so great that, in some cases, protandry cultivars may act as protogeny.

3.3. Types of dichogamy

Walnut trees show two types of dichogamy. Cultivars or genotypes whose male flowers mature first are called protandry. Protandry is the most common type of flowering in walnut trees. A large part of Turkish walnuts is protandry. At the same time, protandry characteristic is predominant in California and French walnut cultivars. Cultivars or genotypes whose female flowers mature before the male flowers are called protogynous. Walnuts with this feature are more common in Iran and the central regions of Asia (Sutyemez, 2006; Akça, 2009). Unlike protogynous and protandrous types, there are some cultivars or types whose male and female flowers mature at the same time on the same tree. This situation is known as homogamy. In a study conducted for the determination of promising walnut genotypes and 20 types were determined, it was identified that 11 genotypes were protandry, 5 types were protogynous and 4 types were homogamy in terms of flowering times (Keles, 2014). In addition, in some walnut selection studies carried out in the world, it was stated that the majority of the types showed protandry characteristics in terms of flowering in walnut types examined. (Rouskas et. al., 1997; Koyuncu and Görgün, 2003; Kaymaz, 2005; Oğuz and Aşkın, 2007).

3.4. The importance of dichogamy for walnut orchard establishment

Flowering time of walnut cultivars affects yield that should be considered before orchard establishment. Dichogamy is one of the most important issues to be given priority for homogeneous and regular yield in the walnut orchards (Shu-Ganga et. al., 2011). In the planning of the walnut orchard, it is necessary to coincide with the period when the female organs of the cultivar to be considered as the main cultivar are receptive and the period when the male organs of the cultivar to be considered as pollinators distribute pollen. For this reason, using more than one cultivar in the planning of a walnut orchard will eliminate the pollination problem and provide regular yield. Otherwise, it should be expected that pollination will be done by pollen from different orchards or by artificial pollination. This situation will either lead to a decrease in productivity values or an excess of labor. Keeping an average of 5% pollinator cultivars in a walnut garden is important for a good fruit set. As shown in Figure 4, the opening of the stigmas at an angle of 45° in the female organ of the main variety and the red color of the tips give clues that they accept pollen. During this period, the male organs of the pollinator cultivar should also change color and be ready for pollen distribution as shown in Figure 6/D. In this case, pollination and fertilization will occur as a result, an optimum fruit set will be achieved.

Depending on the ecological characteristics of the regions where they are located, the opening times of male and female flowers of walnut trees may be different. Different researchers working on walnuts have determined the flowering times of some cultivars according to their regions (Cosmulescu et. al., 2010; Cosmulescu and Botu, 2012; Keles, 2014). Farmers who will grow walnuts can also choose pollinator or main cultivars according to these tables. In Figure 7, male and female flowering times of some walnut varieties found in California ecological conditions are shown.

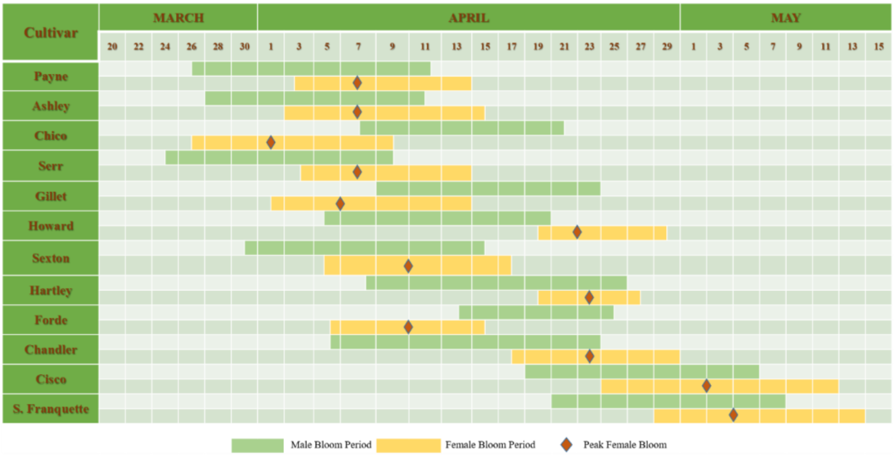

Figure 7. Maturation times of male and female flowers in California (USA) /modified from original (Anonymus, 2021g)

In the table prepared by İbrahim BALKAL, who is known for his studies on walnuts in Turkey (Figure 8), the maturation dates of male and female flowers of some American, French and Turkish walnut varieties grown in Turkey are shown.

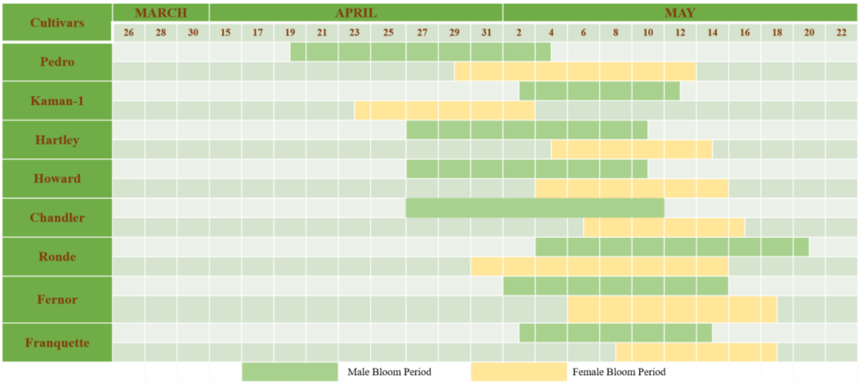

Figure 8. Maturation times of male and female flowers in Inegol-Bursa/ Turkey (Anonymus, 2021h)

Considering the tables before mentioned and similar tables, when establishing a walnut orchard, cultivars in which the maturation times of male and female flowers coincide can be selected. In this way, one of the most important steps for optimum and regular efficiency is taken.

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Part III

The Use of Rootstock in Walnut Cultivation

Dr. Remzi UGUR[1]

1.Introduction

Fruit trees can be described as the combination of two different plant section known as rootstock and scion through grafting. Although many years have been passed, the textural difference between these two different plant parts is immediately understandable with careful examination. It is very important for the rootstock as the soil part of the plant to have a very vigorous and healthy root system (Gundesli et al., 2018; Sharma et al., 2020). Walnut rootstock is usually obtained from seeds called the seedling rootstock. In recent years, thanks to breeding and biotechnological methods developed, rootstocks that can be propagated clonally in walnuts have also been obtained. To establish more homogeneous gardens, we see that clonal rootstocks are more preferred in recent years. Another reason why clonal rootstocks are preferred is that they provıde resistance to biotic and

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abiotic soil conditions in terms of breeding aim. However, when a garden will be established, it is necessary to know the characteristics of rootstock and the variety (Ozcan and Sutyemez, 2017). Thanks to the advances in agricultural biotechnology in recent years even although vegetative propagation of the later developed varieties is carried out *in vitro*, it is very important to know that rootstock breeding has a different process and aim than breeding variety. Sometimes, it is possible to obtain trees that are clonally propagated from standard walnut varieties and formed on their roots. However, this situation has not been an obstacle to the breeding of rootstocks dependent on extreme soil conditions.

1.2 Walnut Rootstock Breeding Studies in the World

Although the use of rootstock in walnut cultivation varies according to the region of cultivation, it has mostly been in the form of seedling rootstocks. Since the beginning of the 1900s, selection breeding studies have been started on the use of walnut rootstocks in the United States and Europe (especially France). With these studies, the use of suitable rootstocks for walnuts began to spread.

In the 1960s, clonal rootstock production started due to reasons such as establishing more homogeneous walnut orchards, increasing yield and quality values, and resistance to soil-borne diseases and pests. Today, although seedling rootstocks are used in walnut orchards, there is a rapid increase in the use of clonal rootstocks (Kluepfel et al., 2015; Granahan et al., 1987).

Towards the end of the 1800s, in the United States of America, California, the use of J*uglans regia* rootstocks brought from France as rootstocks in the walnut orchards started, and by 1912, grafting, that is, the use of rootstock in walnut cultivation, started to be recommended. However, in the following years, rootstocks originating from *J.regia* started to be left gradually due to the developmental differences in the grafted walnuts and the problems in the adaptation ability of these rootstocks (Granahan ve Forde,1985). In studies on species and hybrid tests, the use of *Juglans hindsii* rootstocks as an alternative to *J.regia* has been an increased. After the 1950s, *J. hindsii* rootstocks have been used in almost all gardens in California. However, until the end of the 1800s, hybridization studies were started between *J.regia* and *J. hindsii* species, and garden trials were started on Paradox rootstocks, the first generation hybrid rootstock obtained from these hybridization studies (Potter et al., 2002). After these crossing studies, Paradox rootstock, a hybrid of *J.regia* and *J. hindsii*, was bred. During the passing time, these rootstocks have been used in almost 80% of the walnut orchard in America. The hybrid rootstock Royal (*J.regia* x *J. hindsii*) which has the same parents as Paradox, has a certain cultivation area, but it is not as widespread as Paradox. Since Paradox rootstocks have vigorous and disease resistance than their parents, they have been easier to spread. Although Paradox provides resistance to some diseases such as Armillaria and *Phytophtora*, the idea of breeding resistance to other root diseases and blacklines has become widespread. For this reason, the breeding of rootstock resistant to blackline disease has been started in California (Browne et al., 2015). At the end of these studies, rootstocks that can also be produced vegetatively (Vlach, RX1 and VX211) were bred. Some walnut growers that some rootstocks from English walnuts are resistant to blackline disease and have started to use them. From these three rootstocks bred, although Vlach showed vigorous growth, disease resistance was not determined, RX1 is a *P.microcarpa* x *J. Regia* hybrid which is resistant to nematodes, and VX211 is a *J. hindsii* x *J.regia* hybrid that is resistant to nematodes (Kluepfel et al., 2011).

Walnut rootstock breeding studies in France were started using J.nigra seeds brought from America between 1920 and 1930. At the end of studies performed, promising rootstocks types were obtained in *J.nigra* and *J.regia* origin walnuts, and it was determined that rootstocks from *J.nigra* origin provide better results than *J.regia* walnuts in poor soils. In addition, in other performed studies related to the rootstock characteristics of *J. Hindsii* walnut, it was not preferred since it could not survive cold soil conditions (Ramos and Doyle,1984; Bernard et al., 2018). In the following years, some *J.nigra* ve *J.regia* walnuts selected in France were left to open pollination, and in the studies carried out the hybrids obtained them, hybrid of *J.nigra* x *J.regia* which are more resistant to Armillaria than *J.regia* and has the same growth strength as Paradox rootstocks were obtained. İt is estimated that Paradox rootstock will be difficult to replace *J.nigra* x *J.regia* hybrid rootstocks in France as it is more sensitive to anthracnose. INRA continues to work as the leading research institution in the breeding of walnut rootstock by crossbreeding. Lozeronne, RA 8 611 and Culplat rootstocks are some of them (Ramos,1997; Germain,1999).

Rootstocks breeding studies have been going on for a long time in China where grafting in walnut cultivation based is performed during ancient times. In 2011, The Jin-RS-1 rootstock series was bred and released by Shanxi Academy of Forestry Science. Within this rootstock series, Jin-RS-2 and Jin-RS-3 rootstocks were found to be resistant to cold and soil pathogens in the northern regions of China (Gunn et al., 2010; Han et al., 2016).

Four different walnut cultivars grafted on *J.regia* and *J.nigra* seedling were studied in Hungary. It has been determined that the success of grafting in both rootstock types is around 30-60% and at similar rates. The walnut cultivars Alsoszentivana 117 and Tiszacsecsi 83 performed better on *J. regia* than on *J. nigra*. The success of grafting was 49.00 % in Alsoszentivana 117 variety and 38.2% in Milotai variety grafted on another species J. rupestris. It has been reported that the growth of plants grafted on J. regia species in the garden plant is better than other species (Szentivanyi and Lantos, 1997).

Achim et al., 2007 reported that walnut cultivation is an important product for Romania and the importance of rootstock selection for modern cultivation. For this purpose, he reported that a new seedling rootstock named Portval which was obtained by selection made in the existing natural walnut population was detected. It has been determined that this rootstock shows vigorous growth, flowering type is protandry and resistant to environmental conditions and diseases. It was determined that the growth in the nursery experiment germination rate 80% , the seedling diameter 23.8 mm and the seedling length 160 cm.

In general, seedling rootstocks have been used in Turkey, which is the homeland for walnut genetic resource (Akca and Sutyemez,2016). However, recently, the importance of rootstock breeding has begun to be better understood due to developmental disorders caused by seedling rootstocks and susceptibility problems to some diseases such as phytophthora (Avanzato et al., 2014). In rootstock breeding studies, drought, salinity, and lime resistance should also be considered. Although clonal rootstock breeding studies have just begun in Turkey, it is noteworthy that Paradox and Vlach rootstocks have been used recently.

An improvement work with drought resistance and stunted growth was started in Iran after 2003 year. Educational studies continue by the Tehran University Walnut Research Center (Atefi,1993; Arzani et al.,2008)

2. Rootstock Types

2.1 Rootstocks

The use of rootstock suitable for walnut cultivation can be created between species as well as in-species hybrids. The lack of desired characteristics in a single species has led researchers to use interspecies hybridization (Rom and Carlos,1987). İt is thought that suitability for clonal production, which is the most important feature in rootstocks breeding, can be obtained more easily with interspecies hybridization. Until today, the use of seedling rootstocks was more common in walnuts. These rootstocks include other species within the genus *Juglans*, including *J.hindsii*, *J.regia* and *J.nigra* (McGranahan et al., 1986).

2.1.1 *J.hindsii*

It has been the most widely used rootstock for walnuts among the black walnut seedlings of Northern California (Figure 1). On its narrow leaves, approximately 15-19 leaflets per leaf, 2.5-3.5 cm wide, firm and rough fruits are known. This walnut species, which was first found by Hinds in Sacramento in the USA, was later identified botanically as *Juglans californica* var *hindsii* by researcher Jepson (Rom and Carlos,1987). It has been reported that the main reason for this definition is that *J.hindsii* and *J.californica* are essentially the same species, and the differences are due to climate and soil conditions (Brinkman,1974). *J.hindsii* is in danger of extinction because it can easily pollinate with other walnut species. *J.hindsii* is recommended as a rootstock in deep and fertile soils. There are problems in places where there is a danger of nematode and blackline disease (Sen,1986; McGranahan and Leslie,2012). It is reported that these rootstocks, which are resistant to armillaria and root cancer, do not perform well in high pH and calcareous soils (Woeste et al., 1996; Sen,1986; Gregory et al., 2015; Ramesh et al., 2021).

A- *Juglans hindsii* tree in its natural environment; B-*Juglans hindsii* fruit

Figure 1. Tree and fruit of the genus *Juglans hindsii* are grown in their natural environment.

2.1.2 *J.regia*

J.regia is the most commonly used rootstock in Turkey and in many countries of the world where walnuts are grown. *J.regia* has a smooth gray color with a soft shell structure, the number of leaflets varies between 5 and 9 and has moderately rough fruit. The stratification time of *J.regia* seeds is about eight weeks on average (Rom and

Carlos,1987). However, as the uniformity in the seed deteriorates, the yield from seed stratification will gradually decrease, and there will be differences in the germination of seeds in a certain period time (Brinkman,1974). It would be beneficial to perform homogeneous germination tests for a suitable *J.regia* seed rootstock. There is a possibility that Cherry Leaf Roll Virus (CLRV), which causes blackline disease, can be found in *J.regia* seeds. Although *J.regia* is generally not affected by blackline disease, it is very sensitive to Armillaria, Phytophthora, root meadow nematode, water-saturated soils and salinity (Sen,1986; McGranahan and Leslie,2012). The resistance of *J.regia* to blackline disease began to be widely used in California, especially in Oregon, in the 1950s. For this purpose, they have started breeding studies by selection from genetic sources created by planting *J.regia* seeds brought from outside the USA, especially from China. When comparing some selected rootstock candidates with *J.hindsii*, it was observed that *J.regia* rootstocks formed more vigor crowns. In another study conducted in California, it was determined that some walnut varieties grafted on *J.regia* rootstock were lower in terms of yield efficiency (kg/cm2) than those grafted on *J.hindsii*, *Juglans microcarpa* and *J.major* rootstocks.

Although *J.regia* is used as a common rootstock in many parts of the world, there is almost no rootstock that can be defined as a true seedling rootstock through selective breeding from *J.regia*. In Turkey, in this sense, some saplings in the Marmara region still use some *J.regia* seedlings, which they believe to develop homogeneously and form hairy roots, as rootstocks. In addition, the use of rootstocks from some standard varieties such as Maraş-18 is common in our country. However, since the *J.regia* species is very rich in genetic variation, it is quite possible to develop more effective rootstocks with appropriate rootstock breeding studies. However, since the *J.regia* species is very rich in genetic variation, it is quite possible to develop more effective rootstocks with appropriate rootstock breeding studies (Woeste et al., 1996; Sen,1986; Gregory et al., 2015; Ramesh et al., 2021).

A-*Juglans regia* tree in its natural environment; B-*Juglans regia* fruit

Figure 2. Tree and fruit of the genus *Juglans regia* are grown in their natural environment

2.1.3 *J.nigra*

The homeland of *Juglans nigra*, known as the eastern black walnut, is naturally distributed in the region between New Hampshire-Georgia-Minnesota and Texas in the region from the Middle East-Western parts of the United States to the south. *J.nigra* has been brought to Europe especially for timber since the early 1600s. Since it is resistant to cold in Europe, it has spread to Scandinavian countries. *J.nigra* forms tall trees with 15-23 leaflets. Its fruits are 3-4 cm in diameter with irregular stripes (Rom and Carlos,1987). Compared to *J.regia*, the seed stratification period takes longer (12-16 weeks). *J.nigra* has been used as a rootstock in the United States because it is easy to find (Brinkman,1974; Ozcan and Sutyemez,2017). While walnuts grafted on *J.nigra* formed smaller crowns, they were found to be more resistant to cold, nematodes, Armillaria and *Phytophtora* than walnut varieties grafted on *J.regia* (Kluepfel et al., 2011). However, it was determined that the yield efficiency of rootstocks grafted on J.nigra rootstocks was lower. Although *J.nigra* rootstocks are more sensitive to blackline disease than *J.regia* rootstocks, they are still used in Europe and America as a suitable parent in crossing studies due to their resistance to some other diseases mentioned above and their unique superior characteristics (Gregory et al., 2015; Ramesh et al., 2021).

A- *Juglans nigra* tree; B-*Juglans nigra* fruit

Figure 3. *Juglans nigra* tree and fruit

2.1.4. *J.californica*

This type, known as the California black walnut, spreads naturally from the Santa Mountains to the south in the state of California, USA. It spreads in the form of a bush, its leaflets are less in number than *J.hindsii* (11-15 pieces) (Rom and Carlos,1987). Compared to other *Juglans* species, *J.californica* has a shorter vegetation period. Seeds can begin to germinate after 12 weeks of stratification (Özcan and Sutyemez,2017). Due to its weak growth, this species cannıt hold onto the soil very well if it is used as a rootstock. In addition, root rot was frequently encountered in walnut varieties grafted on *J.californica* rootstocks, and its use as rootstock decreased in the following years (Gregory et al., 2015; Ramesh et al., 2021).

2.1.5 *J.microcarpa*

This species, known as Texas black walnut, has chosen Texas and New Mexico as its natural distribution area. This species, which has the most leaflets and the smallest fruit among the *Juglans* species, shows dwarf development in terms of its general feature (Rom and Carlos,1987; Gregory et al., 2015). This species, which forms dwarf trees even in comfortable soil conditions, has successfully performed as a rootstock in soils with high pH, high boron and chlorine toxicity. Although it showed dwarf growth, yield efficiency in walnut varieties grafted on it was the same as other rootstocks.

2.1.6 *J.major*

It is known as the Arizona black walnut and the distribution area of this species is around New Mexico, Arizona, Colorado and Northern Mexico. This species has an average of 9-13 leaves and 2-3 cm diameter fruit with a deeply corrugated rind. This species, which has been tested on rootstock characteristics in different soil conditions, has not been found to have many superior characteristics to *J.hindsii* (Brinkman,1974). However, it will be beneficial to study the rootstock characteristics of this species, which is not selective in terms of soil in later years (Gregory et al., 2015; Rom and Carlos,1987).

2.1.7 Other *Juglans* Species

Juglans show a lot of diversity as a species. In many of these species, studies on rootstock traits are still ongoing. *Juglans mandshurica* is a native plant of North China and intensive studies are carried out on its rootstock characteristics. Particular emphasis is placed on crossing *J.mandshurica* with *J.regia*. Similar hybridization studies are carried out in Argentina between *Juglans australis* x *J.regia*, *Juglans cinerea* x *J.regia* species (Rom and Carlos,1987; Vahdati et al., 2021). However, the intensive use of black walnuts, which grow naturally in the central and southern parts of the United States, in rootstock breeding studies by hybridization would be the right decision.

2.1.8 Hibrids

In modern fruit growing, it is often not possible to find a rootstock that meets the desired criteria in rootstock breeding within a single species. For this reason, interspecies hybridization studies have been initiated. Suitable rootstocks in many fruit species have been obtained by interspecies hybridization. These rootstocks have achieved a very high commercial value and have been in high demand by growers (Rom and Carlos,1987; Gregory et al., 2015; Zhu et al., 2019). There are also a large number of species in the genus *Juglans*. It varies quite widely from the difference of tree vigor to different soil requirements. However, there are also variations in the resistance of these species to different diseases and pests (Gregory et al., 2015; Ramesh et al., 2021). Most of the species in the genus *Juglans* can form hybrid plants by pollinating among themselves. This situation focused on some species, and the previously known superior rootstock characteristics of these species were tried to be increased by interspecies hybridization. In many studies, crosses of *J.hindsii* x

J.regia, *J.hindsii* x *J.nigra* and *J.nigra* x *J.regia* have been performed. At the end of these studies, rootstocks called Paradox ,Royal and Persian hybrids, which are used extensively, were obtained. More detailed analyzes of these rootstocks are still being made since the 1950s when these studies were carried out (Rom and Carlos,1987; Kluepfel et al., 2011; Zhu et al., 2019; Vahdati et al., 2021). Paradox rootstocks were found to be more resistant to *Phytophthora* and nematodes than both parents. Walnut varieties grafted on Paradox rootstocks show more vigorous growth than the same varieties grafted on *J.regia* rootstocks. Due to the susceptibility of Paradox rootstock mostly to blackline disease, it will be important to select this hybrid and search for more suitable clonal rootstocks that can be produced by tissue culture (Ramesh et al., 2021; Vahdati et al., 2021).

2.1.9 Other *Juglans* Varieties.

Research on the rootstock characteristics of *Juglans* species of different genera under the *Juglandaceae* branch has been limited, only studies on the rootstock characteristics of Pterocarya stenoptera to *J.regia* have been conducted in China. Studies have shown that Pterocarya stenoptera gives excessive bottom shoots, this increases even more in anhydrous conditions and shows incompatibility with some *J.regia* walnuts (Vahdati et al., 2021). This genus, which is highly resistant to nematodes, would be beneficial to cross with the *Juglans* genus and it would be more appropriate to investigate the rootstock characteristics of the hybrids obtained.

2.2 Clonal Rootstocks

2.2.1 Vlach

It is a useful rootstock obtained by selection from Paradox hybrids (*J.hindsii* x *J.regia*) and can be produced clonally by micropropagation (Zhu et al., 2019). Walnuts grafted on this rootstock, which is tolerant to root-knot nematodes, show vigorous growth. Although Vlach clonal rootstock varies according to the variety, it forms more vigorous trees than RX1 and VX211 rootstocks (Brown et al. 2010; Baumgartner et al.2013). It has been determined that it has the same water consumption as VX211 in terms of water stress.

2.2.2 RX1

This rootstock is a hybrid (*J.microcarpa* x *J.regia*) rootstock obtained by crossbreeding. It is moderately resistant to *Phytophthora* bred by the University of California and is also tolerant to Agrobacterium (Brown et al. 2010; Baumgartner et al.2013). Walnuts grafted on RX1 have a medium-strength crown, while yield efficiency is quite high. Classic *J.hindsii* x *J.regia* hybrids formless vigorous trees than rootstocks. It can be easily reproduced by tissue culture. It is moderately tolerant of drought and waterlogging.

2.2.3 VX211

It is another clonal rootstock obtained by selection from Paradox hybrids (*J.hindsii* x *J.regia*).

A group of researchers from the University of California and USDA-ARS selection breeding studies from open pollinated Paradox seedlings, taking into account their genetic relationship carried out in 1996. Growth vigor, nematode resistance and vegetative propagation possibilities were taken into consideration in the study (Potter et al., 2002). Only Vlach clonal rootstock was used until the years of this study. Between 1997 and 2002, the production possibilities of a total of 3000 seedlings consisting of 2000 paradox and 1000 *J.hindsii* seedlings by tissue culture and their resistance to nematodes were investigated. Field trials of promising clones obtained from the selections made in these studies were carried out (Buzo et al.2009). It was observed that this rootstock was resistant to nematodes and Phytophthora in field trials and produced vigorous trees (Gregory et al., 2015; Zhu et al., 2019). Compared to the Vlach rootstock, it forms relatively semi vigorous trees but forms more vigorous trees than the RX1 rootstock.VX211 rootstocks can be easily produced by micropropagation.

2.2.4 Grizzly

It is a random seedling that has drawn attention with its healthy development in a closed walnut garden in the United States and has been bred as a clonal rootstock with studies (Gregory et al., 2015; Vahdati et al.,2021). As a result of more than 20 years of studies on this rootstock, it has been determined that it forms very healthy and vigorous trees in different soil conditions.

A- Walnut trees grafted on paradox rootstock; B- Royal rootstok grafted *Juglans regia* (Preece and Granaham,2015)

Figure 4. *Juglans nigra* tree and fruit

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Part IV

Soil Management and Fertilization in Walnut

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1. Introduction

Walnut, which has been produced since the Neolithic age, is one of the important hard-shelled fruit species with a wide spread area in the world. Among many species included in the genus Juglans in the family Juglandaceae, Juglans regia L. is the most important species traded due to its superior fruit characteristics (McGranahan and Leslie, 1991; Kafkas et al., 2020; Guney et al., 2021). According to the data of 2019, walnut is among the first three with a share of 9.3% among the hard-shelled fruits in the world, and in terms of production amount, it ranks first with a share of 25.8%. China covered 56.1% of the total world walnut production in 2019. Turkey, on the other hand, ranked fourth in walnut production with a share of 5.0%. China accounts for 48.4% of walnut production areas, the USA for 11.3% and Turkey for 9.5% (Anonymous, 2021a). It is grown in many provinces of Turkey (Şen, 1986); (Şimsek, 2010); (Akça, 2012). In

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this respect, our country is among the most important countries in the world in terms of walnut production.

Walnut is a plant that adapts well to different climatic conditions, the need for vernalisation varies between 400-1800 hours, it also grows in locations up to 1700 m high from the sea (Akça 2012).

Walnut, which is an indispensable food in terms of human health due to its nutritional value, has not achieved a significant increase in both the yield per tree and the amount of production despite the high demand today, and even serious problems have been encountered (Oğuz et al., 2016; Okatan et al., 2021). Although many selection studies have been carried out in our country from the past to the present, the existence of a limited number of studies on applications to increase quality and yield in standard varieties is noteworthy. In this respect, conscious fertilization applications can be effective in perennial species such as walnuts. It has been reported that plant nutrient deficiency causes problems such as low yield and internal shrinkage and darkening, which are important for walnuts (Şen, 1986). In commercial cultivation, it is stated that macro and microelements are significantly effective in applied plant feeding programs (Yıldız et al., 2007). In a study conducted in the Niksar district of Tokat, it is stated that the leaf samples taken from 72 walnut orchards have deficiencies in N, P, K, Mg, Ca, Fe, Zn, Mn and Cu (Adıman, 2013). In a study conducted in 46 walnut orchards in Tekirdağ, where nutritional status was determined, it was determined that most of the soils were insufficient in terms of N, P, K, Ca, Mg, S, Fe, Cu, Mn and

Zn nutrients (Solmaz, 2014). Yıldız and Uygur (2016) determined that 61% of walnut orchards in Uşak province had lime problems, 69% had phosphorus, 78% were poor in magnesium, 96% were zinc and 98% were potassium poor. In addition, according to the results of the leaf analysis, they reported that 29% of the walnut orchards had phosphorus, 76% potassium, 80% magnesium, 78% iron, 94% zinc and copper deficiencies. In 157 soil samples collected from the orchards where walnut cultivation is carried out in Mersin province and its districts, it was determined that the orchards were poor in terms of P and K (Arslan and Aydın, 2017). Direction and Sönmez (2021) found the N, P, Cu and Zn contents insufficient, according to the results of leaf analysis in their study in walnut gardens in Burdur region. In addition, they stated that they determined excessive and high lime in soil samples collected from 0-30 and 30-60 cm depths and that this could cause some problems in terms of cultivation and nutrition. Again in the same study, they reported that the organic matter contents of the soil samples were with low hummus and poor, partially insufficient in terms of available P, and available Fe and Zn contents were insufficient.

Mills and Jones (1996), of some macro and micro plant nutrient levels that should be in walnut leaves are N: 2.47-2.98%, P: 0.16-0.24%, K: 1.32-1.47%, Ca: 1.90-2.01%, Mg: %. 0.51-0.63, S: 1500-1600 mg kg^{-1}, Fe: 69-129 mg kg^{-1}, Mn: 207-274 mg kg^{-1}, Cu: 10-12 mg kg^{-1}, Zn: 33-55 mg kg^{-1} and B: 66-81 mg kg^{-1} they reported that it should be in the ranges of. Soil fertility levels of walnut orchard should be in range of pH 6.5 to 7.2, 2.0 to 3.5% OM, 0.25 to 0.3% N, 27.2 to 36.3 kg da^{-1} P, 102.2 to124.9 kg da^{-1} K, 908 to 1360 kg da^{-1} Ca, and 113.5 to 136.2 kg da^{-1} Mg. However, it was also stated that in most cases, the correlation between nutrient supply in the soil and tree growth and nut production is poor (Ponder, 1981). Walnut trees are not a very selective fruit type in terms of soil. It grows better in deep, low ground water, permeable, not holding much water, well-drained, well-ventilated, keeping its moisture, organic matter-rich, alluvial soils (Kramer, 1983); (Anonymous, 2008a); (Anonymous, 2008b); (Anonymous, 2021b).

2. The Importance of Soil Fertility in Walnut Cultivation

Although walnut is a more tolerant plant in terms of soil fertility, it shows significant increases in the amount and quality of the product taken per tree in highly fertile soils. In walnut cultivation, it is necessary to consider the fertility parameters of the soil when establishing a new garden. These parameters are:

- Soil depth,
- Soil texture (water holding and aeration capacities of the soil),
- Soil reaction (pH),
- Soil electrical conductivity (EC),
- Soil lime ($CaCO_3$),
- Soil organic matter (SOM) and
- Soil is plant nutrients (macro and micronutrients and their amounts).

2.1. Soil Depth

For the walnut plant, generally deep and well-drained soils are defined as suitable soils. In studies in the USA, a soil depth of at least 90-100 cm is recommended for restrictive and unsuitable soils (Losche, 1973); (Funk, 1979). According to Ponder (2004), effective soil rooting depth should be 90 to 150 cm or more and should not be limited to a layer of sand or gravel, compacted massive clay layer or bedrock. However, it has been detected that walnut roots can also benefit from deeper soils and absorb water at a depth of 300-370 cm (Ponder, 1981). Walnut trees grow best in soils where their roots can easily grow up to 2.5-3 m. For this reason, 3 m below the soil should be checked before the garden is established (Anonymous, 2021c). Considering that walnut roots can go down to 3-3.5 m, it requires deep soil for a good cultivation. Soil depth should not be less than 1.5-2 m. For this reason, when considering the establishment of an economic garden, it is useful to know the soil depth and the underlying soil structure (Vural, 2009).

2.2. Soil Texture

Texture is the size limitation of the sand, silt and clay that make up the soil. It affects root performance by inhibiting the soil's water holding capacity, aeration, plant growth and in some cases, plant nutrition (Ponder, 1981); (Begg, 1985). Although walnut trees are not selective in terms of soil, they grow well in deep soils with a ground water level of 2.5-3.0 meters, which do not hold water, maintain their moisture. It can not grow in water-retaining clay soils and stagnant watery places. Walnut roots are taprooted and since they grow into the deep, soils with moist undersides, deep and loam, silty loam, silty clay loam, silty, clay loam, sandy loam and fine sandy loam are more suitable for cultivation. Excess moisture and stagnant water prevent the roots from getting the necessary oxygen, so root growth slows down and the tree stops growing (Ponder, 2004); (Anonymous, 2021d).

2.3. Soil Reaction (pH)

Soil reaction (pH) is one of the most important properties of the soil solution. The pH of the soil causes the movement of plant nutrients, their accumulation in the soil or making them unavailable, preventing them from being uptaken by the roots or hindering growth and development by making toxic effects. Fruit trees develop best in soils between pH 5.5–6.5. However, soils with a pH of up to 8.5 are also used in fruit cultivation (Arslan and Aydın, 2017). For walnuts, soil pH is required to be 6.5-7.2 (Ponder, 2004); (Anonymous, 2021b). In a study they conducted in the Burdur region, Yön and Sönmez (2021) reported that the pH of walnut-grown soils varied between 6.58-7.59 at 0-30 cm and 6.84-7.64 at 30-60 cm depth. In another study, it was stated that the pH value of the soil should be slightly acid and neutral (6.0-7.5), and that iron and zinc deficiency are generally observed in the young leaves of the trees in walnut orchards established in soils with high pH value (more than 8) (Anonymous, 2021e).

2.4. Electrical Conductivity (EC)

Electrical conductivity is directly related to salinity. Walnuts are also in the moderate fruit group in terms of resistance to soil salinity (Anonymous, 2021c). Since walnuts are sensitive to salinity, the amount of salt in the soil and irrigation water must comply with the specified standards. As salinity increases, yield loss also increases (Sesli, 2014); (Anonymous, 2016). Values of 1.5 dS m^{-1} and below are suitable for walnut cultivation. If the EC is higher than 1.7 dS m^{-1}, yield loss begins. When EC is 2.3 dS m^{-1}, 10%, when EC is 4.8 dS m^{-1}, there is a 50% efficiency decrease (Akça, 2000); (Anonymous, 2021f).

Root, trunk and shoot length of walnut trees exposed to saline stress, in leaf area and number, significant decreases were observed in the amount and yield of chlorophyll. When the plant was exposed to salinity stress for a long time, it was observed that signs of ion toxicity and water deficiencies on old leaves, and carbohydrate deficiencies appeared in young leaves (Meddamaz and Ellialtıoglu, 1994); (Sivritepe, 1995).

2.5. Soil Lime ($CaCO_3$)

Especially in the form of $CaCO_3$, the presence of calcium more than desired causes many plant nutrient elements to be bonded. For this reason, it is desirable to have the appropriate amount of calcium in the soil. The effect of lime on the walnut plant, which is more tolerant to soil lime than other fruit species, is indirect due to relationship of walnut with pH (Arslan and Aydın, 2017). In walnut orchards established on very calcareous soils, iron and zinc deficiency are usually seen in the young leaves of the trees (Anonymous, 2021e). At the same time, it is known that phosphorus precipitates in the form of tricalcium phosphates in very calcareous soils and turns into a form that the plant cannot uptake. Direction and Sönmez (2021), in a study they conducted in walnut orchards in Burdur region, reported that more than 80% of the soils were high and extremely high in lime, and that the Fe and Zn contents, and partly phosphorus contents, were insufficient in all of the soils. Arslan and Aydın (2017), in their study in districts of Mersin, where walnut cultivation was carried out, reported that approximately 50% of the soils were too calcareous and also 60% were low in phosphorus. Fe deficiency is often called lime chlorosis (Carlson, 1985).

2.6. Soil Organic Matter (SOM)

Organic matter is very important for both the nutrition of the tree, root health and drainage. Walnut grows well in soils rich in organic matter content. The amount of organic matter desired in the soil in walnut cultivation is 2.0-3.5% (Ponder, 2004). Therefore, it is necessary to increase the organic matter content of the soil by applying suitable farmyard manures. However, increasing the amount of organic matter of the soil with farm fertilizers is both expensive and if poorly burned manure is used, weeds and some soil diseases and pests, if any, are infected to the soil. The most economical and optimal way to increase the amount of organic matter is to make a green manuring program.

Green manure plant to be determined in accordance with the region, preferably leguminous plants such as vetch, broad bean and sainfoin, are grown between rows from the beginning of autumn to the spring period, and green manuring is made by mixing the plants with the soil in the middle of the flowering period. Since there will be no weed growth between rows by growing green manure plants, weed control may not be necessary (Anonymous, 2021g).

2.7. Plant Nutrient Elements

Plant nutrients consist of some chemical elements. Therefore, the nutrients needed by green plants are inorganic. Plants uptake inorganic nutrients through their roots or leaves. Plants need at least 17 plant nutrients to ensure their normal growing. Three of these elements are carbon, hydrogen and oxygen. Since these elements are mostly uptaken from air and water, they are considered as non-mineral plant nutrients. In addition, they are almost ignored in plant nutrition due to their sufficient resources (Jones and Jacobsen, 2001); (Fageria, 2009); (Kacar and Katkat, 2010). Plants uptake 14 other essential elements (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron Fe), manganese (Mn), copper (Cu) , zinc (Zn), boron (B), molybdenum (Mo), chlorine (Cl) and nickel (Ni)) directly from the soil. Elements can be taken by plants in the form of anions and cations, as well as in the form of molecules. The proportional amounts of these soluble elements are also different from each other (Bolat and Kara, 2017). In perennial plants such as walnuts, it is necessary not only soil analysis, but also leaf analysis to find out the nutritional status of the plants. It is of particular importance to interpret the soil and plant analyzes together for walnuts. Balanced fertilization is essential to increase walnut yield and quality (Ponder and Schlesinger, 1986); (Garrett et al., 1991); (Jones et al., 1995); (Jacobs et al., 2005).

2.7.1. Uptaking Soil and Leaf Samples

Upaking soil samples is closely related to climatic conditions. Soil samples can be collected at any time during the year if the humidity and temperature conditions are suitable. However, in order for the analysis results to be in the hands of the farmers on time, soil samples should be collected 1.5-2 months before planting or fertilizing. Before the soil samples are collected, a sketch of the land is drawn if the land is very large and showing differences. In this sketch drawn, the differences are indicated. Different samples are taken from each different region. Soil samples are collected from 10 different points by walking in a zig-zag (S or Z shape) on the land where the walnut garden will be established. Soil samples are collected from 20-40-60 cm depths of these points separately. If necessary, samples are also taken from 90 and 120 cm depths. For leaf samples, middle leaflet pairs are collected from mature shoots with 5, 9 or 13 leaflets 6-8 weeks after the full flowering period (in July or August) from at least 25 trees (Kacar and İnal, 2010).

2.7.2. Fertilizer Application

For a fertilization program to be carried out in walnut orchards, soil and leaf analyzes must be done. The results from leaf samples are important in determining the amounts of fertilizer to be given. Fertilization in walnuts, 1. Fertilizing when establishing a garden, 2. Sapling fertilization, 3. Fertilization in the juvenil period and 4. Four separate periods are taken into account as fertilizing during the full yield period. In addition, when necessary, foliar fertilization can be carried out as well as from the soil. In general, fertilization is carried out in two different periods in walnuts of both in sapling age and in full yield age. The first fertilization is carried out 2-3 weeks before the bud swell on the shoots at the beginning of spring. This fertilization is called base dressing. The second fertilization is applied before irrigation (in the period between fruit set and fruit growth) and watered. This fertilization is called top dressing.

2.7.2.1. Fertilizing when establishing a garden

Fertilizing when establishing a garden, which is the fertilization applied to the whole area or to planting pits without planting saplings. According to the results of soil analysis; farmyard manure, phosphorus and potassium fertilizers should be used. In addition, liming or sulfur applications should be carried out according to the status of pH and organic fertilization also according to the state of organic matter into the sapling planting pits. In cases where organic fertilizers of animal origin are insufficient, green manure crops can be sown to interrows after planting saplings. Since walnut trees grow well in light-textured alluvial soils, the soils are generally poor in phosphorus, potassium and magnesium. Saplings are planted in pits containing a mixture of 100-150 g of Normal Super Phosphate and Potassium Sulphate fertilizers, 50-60 g of Magnesium Sulphate and 2-3 g of Zinc Sulphate. Then, the top soil is mixed with barnyard manure and the pits are filled. According to irrigation methods after planting saplings, a fertilization program is applied until the saplings reach the age of yield (Anonymous, 2021b and h).

2.7.2.2. Sapling fertilization

Walnut saplings become yielding when they reach 4-7 years of age, depending on the variety. During this period, it is necessary to fertilize the saplings in a way to ensure that they have a strong root structure and healthy development of the above-ground part. Fertilizers given to the crown projection of the saplings or to the part where the drip irrigation pipes pass (60-70 cm) are mixed to a depth that will not cut the roots. In base dressing, all of the phosphorus and potassium and half of the nitrogen are given according to the soil analysis result. The other half of the nitrogen is applied 1-2 times before irrigation (fruit set-fruit growth) and irrigated. In fertilization with drip irrigation, 30% of nitrogen fertilizer, 60-70% of phosphorus fertilizer and 50% of potassium fertilizer are applied during the bottom fertilizer application period. The remaining part of the fertilizers is applied in each irrigation in accordance with the irrigation program with fertilizers suitable for the drip irrigation system. The amounts of fertilizers that should be given according to the age of the sapling are given in Table 1.

Table 1. Fertilizer Amounts to Be Given According to The Age of the Saplings (Anonymous, 2021g).

Growth Period	Fertilizer Type	Sapling Age (g fertilizer/sapling)		
		1	2	3
Bottom Fertilizer (before bud swelling on shoots)	15-15-15	400	800	1000
Tassel Fall Period	Ammonium Sulphate	100	200	200
Fruit Set-Fruit Growth	Calcium Ammonium Nitrate (CAN)	-	-	100

2.7.2.3. Fertilization sapling in the juvenil period

Nitrogen is the most needed nutrient element of walnut saplings, which are in the juvenil period, in the spring flowering and shoot formation period. The amounts of fertilizers that should be given to saplings of the age of yield (in the juvenil period) are given in Table 2.

Table 2. The Amounts of Fertilizers to Be Given to The Saplings During The Yielding Period (Anonymous, 2021g).

Growth Period	Fertilizer Type	Sapling Age (g fertilizer/sapling)		
		4	5	6
Bottom Fertilizer (before bud swelling on shoots)	15-15-15	1000	1200	1200
Tassel Fall Period	Ammonium Sulphate	200	300	300
Fruit Set-Fruit Growth	Calcium Ammonium Nitrate (CAN)	200	200	200

2.7.2.4. Fertilizing in fully yielding walnut trees

Depending on the light, medium and heavy texture of the soil, bottom fertilizers are mixed into the soil at a depth that will not cut the roots and at the distance of the tree's crown projection. Top dressing should be pre-precipitation. Fertilizer should be used according to the soil analysis and the amount of product that can be taken per tree. In Table 3, amounts of fertilizer that should be given according to the amount of product that can be taken per tree is given. Composite or potassium fertilizers containing chlorine should not be used on walnut trees that do not like salinity. Base dressing with potassium sulfate should be preferred for the increase in %fat, %protein and aroma in the edible part of walnuts.

Table 3. Amounts of Fertilizer to Be Given According to Amounts of Product That Can Be Taken Per Tree (Anonymous, 2021g).

Growth Period	Fertilizer Type	Yield (kg/tree, g fertilizer/tree)			
		10-20	20-40	40-60	> 60
Bottom Fertilizer (before bud swelling on shoots)	15-15-15	1500-2000	2500-3000	3500-4000	4500-5000
Tassel Fall Period	Ammonium Sulphate	600	800	1000	1200
Fruit Set-Fruit Growth	Calcium Ammonium Nitrate (CAN)	400	500	600	800

2.7.2.5. Fertilization according to the drip irrigation system

Base Dressing: 10 kg of 15-15-15 fertilizer per decare is divided by the number of trees per decare and given to the part where the drip irrigation pipes pass and mixed with the soil. The amounts of fertilizer given for one-year-old saplings in Table 4 should be applied by increasing by 15% each year until the saplings reach the full yield period.

Table 4. Amounts and Application Intervals of Fertilizers to Be Given by Drip Irrigation for One-Year-Old Saplings (Anonymous, 2021g).

Growth Period	g fertilizer/acre/month				
	15-15-15	MAP	KNO_3	$ZnSO_4$	MKP
April	500	500	500	50	-
May	832	1500	1000	100	-
June	664	1000	1500	100	-
July	332	500	1500	50	-
August			1000		-
September					3

MAP: Monoammonium phosphate, KNO3: Potassium nitrate, ZnSO4: Zinc sulfate, MKP: Monopotassium phosphate

All fertilizers and doses given in this study are for exemplary purposes. The main thing is the recommended fertilization program according to the results of soil and plant (leaf) analysis.

2.7.2.6. Foliar Fertilization

Foliar fertilizers can be in solid (powder and crystal) and liquid form. Leaf fertilizers in liquid form are generally obtained by dissolving solid form mineral fertilizers in water, acid and special solutions and preparing them as concentrated solutions. The total amount of active substances is 2-3 times less than those in solid form. The application dose in these fertilizers should be 1-2% in macroelements and 0.1-0.2% in microelements on the basis of effective substances. It should not be used in windy weather and before precipitation. Since very little of it is taken by the plant, it should be applied 2-3 times at certain times (Aslan, 2013). Application doses and times depend on the growth period of the plant and the thickness of the cuticula layer in the plant leaf. It should be applied in the proportions written on the package. In case of excessive use, burns may occur on the leaves of the plant. The symptoms and symptoms locations of the deficiency of plant nutrients in walnuts are given in Table 5.

Tablo 5. Plant Nutrient Deficiency Symptoms and Symptom Locations in Walnuts (Anonymous, 2021h).

Deficient Element	Symptom	Symptom Location in the Plant
N, (S), Fe	Chlorosis	Old and Ripe Leaves
Mg, (Mn)	Chlorosis	
Fe, (S)	Chlorosis	Young Leaves
Zn, Mn, (B)	Chlorosis	
K, Mg	Necrosis	Old and Ripe Leaves
Mg, (Mn)	Necrosis	
B, Cu, Cl	Necrosis	Young Leaves
B, Mo, Ca, Zn	Deformation	Young Leaves
P	Dark Gray Color	Very Old Leaves

3. Conclusion

Plant nutrient deficiencies in our soils reveal the importance of plant nutrition practices in the production process. The internal fruit quality of walnut, which is one of the hard-shelled fruit types, is important and is a clear reason of preference for consumers. In order to increase the yield and quality in walnuts (fruit internal weight, width, height and height, yield, shell thickness, internal shrinkage, color values, shoot length, lateral branch efficiency, etc.), a balanced fertilization is required according to the analysis results of soil and leaf samples (Ponder and Schlesinger, 1986); (Garrett et al., 1991); (Jones et al., 1995); (Jacobs et al., 2005); (Adiloğlu and Adiloğlu, 2005).

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Part V

Micropropagation of Walnut

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Walnut has a taxonomy that includes a total of 21 species belonging to the genus *Juglans* from the *Juglandaceae* family. The most important of these species are *Juglans regia* L. *Juglans nigra* L. and *Juglans cinerea* L.. Wild walnuts as natural habitats have spread as far as Alaska in Europe, Asia and North America. They are walnuts belonging to the *Juglans regia* species, which are naturally grown for their nut and investigation subject to the breeding studies. In addition, *Juglans nigra* is grown for its timber. *Juglans hindsii*, which is called California black walnut, is mostly used in rootstock breeding studies (McGranahan and Leslie, 1990). Walnut is a temperate climate fruit. It's wood has been used for a long time due to its high quality, its leaves and fruit skin due to its pharmacological properties, and its fruit

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due to its nutritional properties. The fruit of the walnut is very rich in protein, fat components and energy. Walnut, which spreads naturally on rich and high quality soils and streams, can also form healthy plants in arid and rocky areas (Pijut, 2004).

Walnut species are usually propagated by seeds. Walnut *(Juglans spp*). the dormant embryo reproduction and hybridization method are very effective in the development of high-yielding varieties. Walnut varieties are also propagated vegetatively by grafting onto seedling rootstocks. Different rates of success have been achieved with different intra- and inter-species grafting techniques (Ki and Ding, 1990). Vegetative propagation in walnut cultivation is labor, time wastage and grafting, which is expensive. at the same time, vegetative propagation is a very difficult situation as their rooting ability is low (Land and Cunninham., 1994). They stated that in many studies conducted in previous years, the desired success in walnut production and reproduction is still not achieved, and the reason for this is irregular and low rooting rates (Claudet et al., 1992; Heloir et al., 1996). There are different studies with walnuts for research, clonal reproduction, and genetic development. Progress in progressive techniques for tissue culture production and hybrids of walnuts has been very successful in recent years. Plantlets were obtained through shoot tip propagation, cultured node segments, and somatic embryogenesis (Payghamzadeh, 2008)

The use of grafting and rootstock in walnut cultivation dates back to ancient times. In the beginning years, rootstocks were generally used as seedling rootstocks. Orchards were prepared from walnut cultivars grafted on walnut seedling rootstocks of different species. However, in the following years, the use of clone rootstocks began in order to establish more homogeneous orchards and to provide resistance against some soil pathogens. Hardwood production in walnuts is very difficult and has not been preferred because it provides low success. With the emergence of Paradox rootstocks, micro production possibilities began to be sought in rootstock candidates obtained from these hybrids (Kaur et al. 2006). In fact, micropropagation has become a vejetatif propagation method that has been economically used in many fruit and rootstock varieties by the private sector in recent years. Successful results are obtained especially in the reproduction of stone and pome fruit rootstocks. Many private companies produce rootstock with this method and sell until export. This success achieved in stone and pome fruits could not be achieved in walnuts in the early days. One of the most important reasons for this is that explants taken to *in vitro* secrete phenolic compounds that cause darkening from the cutting site (Rout et al.1999). This situation was seen as the most important obstacle to the micropropagation of walnut. Some researchers have tried to prevent this situation by adding substances such as citric acid, ascorbic acid, activated charcoal, polyvlinyl pyyrolidone (PVP), thirourea, L-cysteine and glutamine to the medium. In other studies, polyphenol activity was tried to be reduced with different dark applications (Payghamzadeh, 2008). In another study, it was concluded that activated charcoal added to the first culture medium was effective. In fact, the biggest problem in walnut micropropagation is the release of Juglon compounds, which are allelochemicals, into the medium after they are taken into the medium. In addition to the above-mentioned applications, it is reported that the problem is solved to a great extent by taking explants to fresh nutrient media at intervals of 1-3-5 days (Driver and Kuniyuki, 1984; McGranahan et al., 1984).

IN VITRO RESEARCH PROTOCOL

In the spring season, the material should be taken from walnut trees that show 2-4 weeks of development, 0.5-0.8 cm in diameter, 2-5 cm between the knuckles and leaf buds. Antioxidant liquor and fungicide liquor should be used for pre-sterilization before the materials are taken to the laboratory, and then they should be processed in the laboratory. In August and September, semi-woody cuttings are taken from walnut trees aged 2-4 years, 20 cm tall. The surface is disinfected by mixing with the fungicide mixture for 20 minutes in the laboratory and then washed three times with sterile distilled water. The cuttings are placed on filter paper until dry and left with 50 ml of water in 200 cc bottles under sterile conditions, then closed with transparent plastic bags. It is kept in the climatic chamber for a 16-hour photoperiod at 25 ± 1 C until vegetative leaf bud formation. Walnut the widely accepted operation includes surface sterilization of beginning explants with 50 - 70% (v/v) ethanol for 20 – 30 s traced by 0.1 - 15% (v/v) sodium hypochlorite implicating 0.01% Tween 20 for 10 - 20 min wait. Later three 5 min washes were in sterile distilled water (Payghamzadeh and Kazemitabar, 2011). Kour et al. (2006), Netar Akhort, Gobind, Solding walnut kinds vis., of the choice kinds made embryo culture in ripe fruit. Ripe fruits were bathed in flowing water faucet water, the epicarp was taken, and stayed fruit piece was sterilized by treating with 0.1% sodium hypochlorite solution and washed twice for 5 minutes with sterile distilled water. San and Dumanoglu (2006), Yalova-1, Şebin, Bilecik, KR-1, KR-2, Sen-2, 07-KOR-1, Tokat-1, Kaman-1, walnut kinds somatic embryogenesis made works. Kaman-5 cultivars were sterilized with 3.75% bleach in the raw fruit. Fruits were sterilized by dip in 3.75% (v/v) sodium hypochlorite for 25minute dip three 5-minute washes in sterile distilled water. Roschke and Pijut (2006); Micropropagation studies were carried out on shoots in walnuts and shoots in leaves. Sterilization was done separately for leaves and shoots. For shoots: shoots are washed under the flowing water for 30 minutes. Later shoots were dissected and dipped in 70% ethanol for 30 seconds and disinfected for 20 minutes in 15% sodium carbonate 0.01% Tween liquor on an orbital shaker. Rinsed in sterile water four times for 30 seconds. For leaves: the surface of the explants was washed in flowing tap water for 5 minutes, and sterilized in 10% sodium hypochlorite liquor for 10 minutes. Later washed four times with sterile water.

A successful micropropagation is studied in four stages, which includes different methods:

1-Sterilization and initial culture,

2-Shoot formation and multiplication,

3- Formation and rooting of microshoots,

4-Hardening and acclimatization of plants from *in vitro* (Payghamzadeh and Kazemitabar, 2011)

1-Sterilization and initial culture

Embryo, cotyledon or axillary buds are used in walnut micropropagation. In recent years, the most commercially used shoot meristem. At this stage, it is very important that the explant is not adversely affected by the sterilization material. A careful sterilization process will also prevent future contamination. The shoots taken from the explant source plant are cleaned with the help of liquid soap and a brush and kept under running water for 2-3 hours. Then it is kept in 70% ethanol for 2-3 minutes and rinsed with pure water. After this stage, the explants are sterilized in a sterile cabinet with 70% Actigen ((Natural Protection System, NPS Biocidal) 100% natural water-based and 0.015% active chlorine containing liquid, floor and surface disinfectant) for 5 minutes and then 5' 3 times. rinsed with sterile distilled water for each minute.

Figure 1. Obtaining meristematic nodal tissue from the explant source

Table 1. Different sterilization methot of explants.

Species/ Cultivars	Explants	Study	Disinfectant and dosage	Reference
J. regia L.cv.	Mature fruit	Embryo culture	0.1% sodium hypochlorite	Kour et al. (2006)
J. regia L.cv.	Immature fruit	Somatic embryogenesis	3.75% sodium hypochlorite	San and Hatic (2006)
J. regia L.	Shoot and leaf	Adventitious shoot regeneration and micro propagation	For shoots: 70% ethanol, 15% bleach solution	Roschke and Pijut (2006)
J. cinerea L.	Nodal segment	Axillary bud culture	0.8% (v/v) sodium hypochlorite (15% clorox bleach)	Pijut (1997)
J. regia L. rootstock	Fruit Nut	Micropropagation Embryo culture	0.5% NaClO, 75% EtOH	Revilla et al. (1989)

2-Shoot formation and multiplication

In many different studies, it has been reported that walnut explants micropropagated very well to appropriate *in vitro* conditions. Axillary buds that have grown in the starting medium are taken into Driver and Kuniyuki Walnut (DKW) medium supplemented with approximately 8-9 µM BAP (Benzylaminopurin). In other studies, it has been revealed that modified MS nutrient media supplemented with BAP at rates ranging from 4.4-8.9 µM can also give successful results for *J.regia*. Sister shoot formation varies highly according to genotypes, even in similar media (Payghamzadeh and Kazemitabar, 2011). Scaltsoyiannes et al. 1997 reported that they obtained different results in micropropagation of 11 different walnut genotypes. Payghamzadeh and Kazemitamar 2010, who made micropropagation experiments in different walnut varieties, reported that the media to be used may vary according to the genotypes.

Micropropagation of walnuts different media by Driver and Kuniyuki (1984) (DKW), Murashige and Skoog (1962) (MS), Cheng (1975), Gamborg et al (1968) and Lloyd and McCown (1981), (WPM), Rodríguez (1982), medium K(h) are used. Among these media, Driver and Kuniyuki (1984) (DKW), Murashige and Skoog (1962) (MS) are the most used in walnut micropropagation. The DKW medium is especially optimized for the production of Paradox rootstocks. Micropropagation of many *Juglans* species is successfully carried out with this medium. However, some researchers reported that they obtained successful results from the MS medium. Saadata and Hennerty 2002 reported that *J.regia* needs more salt minerals in micropropagation, and the most suitable medium for this should be DKW. Although DKW salts are similar to MS in terms of minerals and nitrogen, it has a higher concentration in terms of other ions. Saadata and Hennerty 2002 reported that DKW and MS gave higher results than WPM in terms of callus weight and average shoot length. Driver and Kuniyuki 1984 reported that DKW gave more positive results in the results obtained from micropropagation of Paradox rootstock in DKW and WPM media. In a similar study by the same researchers in black walnut, they found better results in shoot length, shoot thickness, leaf width and color of DKW medium.

MS medium has higher nitrate concentration compared to WPM medium. This situation is thought to cause metabolic stress in plants.

It is reported that this nutrient medium does not cause stress in the plant cell, since many components in the MS nutrient medium are not in WPM. It has been determined that leaf deformations and plant stresses occur in potato plants taken into MS medium due to the cobalt content. WPM does not contain cobalt or nickel. While potassium iodide is present in MS, there is no iodine source in WPM. Some studies have shown that iodine sources have a toxic effect on the micro-propagation of golden weed (Haplopappus gracilis).

Payghamzadeh and Kazemitamar 2008(a) used modified DKW with MT mediums for walnut micropropagation. At the end of the study, they determined that sister shoot formation was higher than DKW medium. Positive results were obtained in embryo germination from the medium developed for walnut micropropagation (NGE) by Murciano de Investigacióny Desarrollo Agrarioy alimentario (IMIDA). Later, researchers compared DKW, WPM and NGE media. The highest germination percentage was obtained in WPM with the highest 81%, then 54% and 62% values were obtained from DKW and NGE, respectively. In the micropropagation of *J.cinerea*, it was revealed that the explants taken in DKW and NGE mediums showed similar growth rates. However, contrary to these results, it has been reported in many studies that DKW medium is most suitable for micropropagation of *J.regia* (Kaur et al.2006).

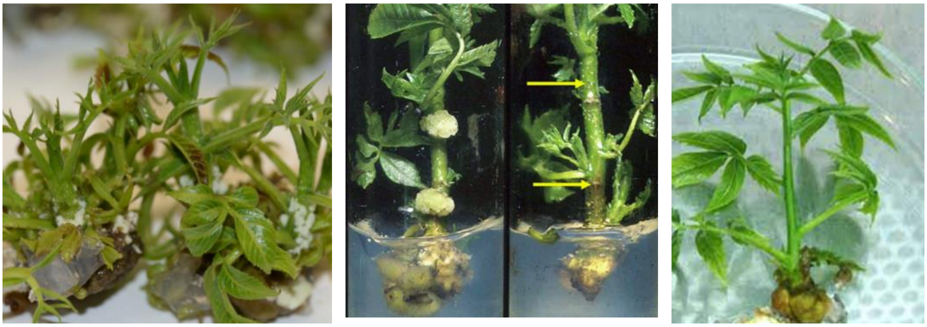

Figure 2. Shoot multiplication of walnut explants

3- Formation and rooting of microshoots

Phenolic compounds and some internal bacterial contaminations are the main barriers to regeneration and rooting in successful in vitro studies of walnut protocol. Many studies on in vitro propagation of some walnut genotypes have reported that these plants bear fruit early compared to genotypes propagated by conventional methods. They also stated that they have a good rooting system and that there is no incompatibility (Lopez, 2004; Nomiya et al., 2004; Vahdati et al., 2009; Zarghami and Salari, 2015). Rooting of walnut micro shoots depends on internal and external factors. In the rooting studies of hybrid walnut rootstocks (*J.nigra* X *J.regia*), 24.6 µM IBA was applied and it was determined that the rooting rate was very high after a 5-day dark period. It has been observed that some internal hormones and peroxidase enzyme activity increase rooting in micro shoots. Leslie and McGranahan (1992) reported that they obtained the highest rooting rate from 4.9 or 24.6 µM IBA application. It has been reported that rooting of microshoots taken to medium with proportionally low salt concentration is higher. Scaltsoyiannes et al. (1997) found that rooting was higher in DKW medium where the macro element was reduced by ¼ and the NO_3/NH_4 ratio was increased from 0.1 to 3.0. Hyndman et al. (1982) determined that DKW medium containing reduced KNO_3 and NH_4NO_3 concentration was more effective in rooting. Long et al. (1995) observed that rooting was higher in DKW medium containing high sugar concentration and reduced nitrogen (456.2 mg/L NH_4NO_3 and 634.0 mg/L Ca $(NO_3)_2$). It has been revealed that rooting rate, average root length and root number are high in all media where activated charcoal is used. In addition, it was revealed that micro shoots taken to the medium with activated charcoal needed less time for rooting.

Revilla et al. (1989) observed that micro shoots that were kept in 2 $mg.l^{-1}$ liquid nutrient medium for 24 hours and then taken to solid nutrient medium with the same hormone ratio rooted more easily. Researchers stated this situation as some polyphenols that prevent rooting on the cut surface of the microshoots were transferred to the liquid medium and then rooting increased in the solid medium taken. Payghamzadeh and Kazemitabar (2008) investigated the effects of different phase, activated charcoal and BAP concentrations on rooting in DKW medium, and reported that double phase nutrient culture provided more effective rooting. Many studies so far have shown that auxin hormones are quite effective in rooting. The best result in rooting can be obtained by:

When the microshoots grow to 4-5 cm, they are taken into DKW medium containing ¼ reduced macro element supplemented with 24.6

μM IBA and 40 g.l-1. Then it is kept at 24 °C for 6 days and at 21 °C for 6 hours. After this application is completed, the micro shoots are transferred to DKW medium containing sterile vermiculite and gelled ¼ reduced macro element. Scaltsoyiannes et al (1997) reported that the use of medium type vermiculite in rooting increases rooting and root quality as it allows the roots to breathe more easily.

Figure 3. *In vitro* rooting of walnut

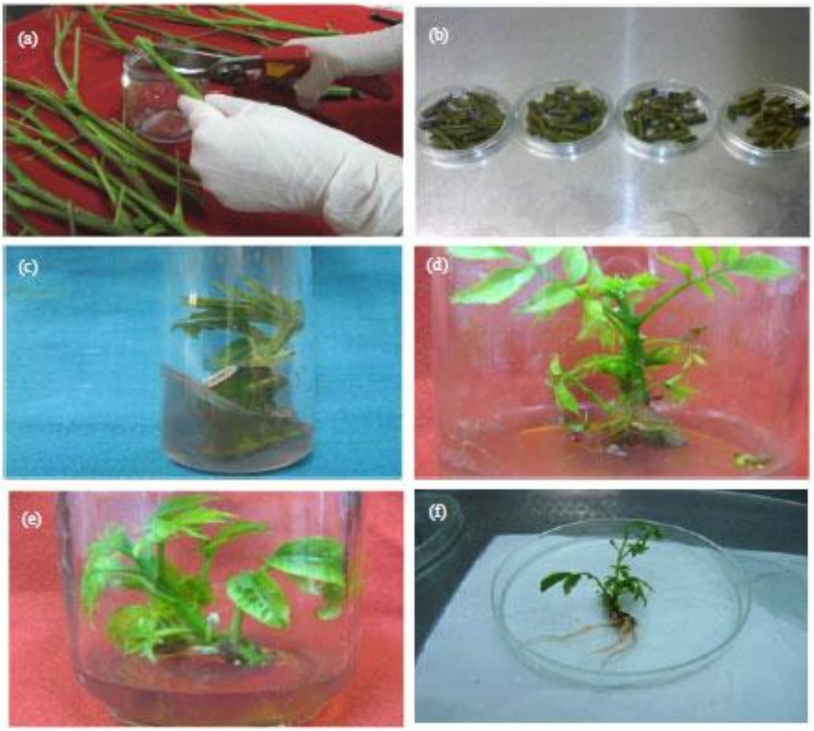

Figure 5. In vitro propagation of walnut (a, b) preparation, (c) Establishment, (d, e) Proliferation and (f) Rooting (Zarghami and Salari, 2015)

Table 2. Salts composition (mg.l^{-1}) of culture medium

Component	**MS**	**DKW**	**WPM**	**NGE**	**B_5**	**LP**
NH_4NO_3	1650	1416	400	908	-	908
KNO_3	1900	-	-	723	2500	-
$Ca(NO_3)_2.4H_2O$	-	1968	556	2249	-	1262
$CaCl_2.2H_2O$	440	149	96	699	150	122.2
K_2SO_4	-	1559	990	-	250	1274.5
$MgSO_4.7H_2O$	370	740	370	2053	134	555
$(NH_4)2SO_4$	-	-	-	-	10	-
$MnS0_4.H_2O$	-	-	-	-	150	27.9

$NaH_2PO_4.H_2O$	-	-	-	-	-	-
KH_2PO_4	170	265	170	155	-	217.5
$MnSO_4.4H_2O$	22.30	33.5	22.3	22.3	0.25	-
$Na_2MoO_4.2H_2O$	0.25	0.39	0.25	0.25	2	0.32
$ZnSO_4.7H_2O$	8.6	-	8.6	8.6	-	4.3
$Zn(NO_3)_2.6H_2O$	-	17	-	-	0.75	8.5
Kl	0.83	-	-	0.83	3	-
H_3BO_3	6.2	4.8	6.2	6.2	0.025	5.5
$CuSO_4.5H_2O$	0.025	0.25	0.25	0.025	0.025	0.25
$CoCl.6H_2O$	0.025	-	-	0.025	-	-
$NiSO_4.6H_2O$	-	0.005	-	-	-	-
$FeSO_4.7H_2O$	27.8	33.8	27.8	27.8	37.3	30.8
$Na_2EDTA.2H_2O$	37.3	45.4	37.3	37.3	100	41.35
Myo-inositol	100	100	100	100	10	100
Thiamin-HCL	0.1	2	1	0.1	1	1.5
Nicotinic acid	0.5	1	0.5	0.5	1	0.75
Pyridoxine-HCL	0.5	-	0.5	0.5	-	0.25
Glycine	2	2	2	2	-	2
Glutamine	-	-	2	-	-	-
Sucrose	30.000	30.000	30.000	30.000	20.000	30.000

Table 3. Commonly used hormones, mediums and explants for *in vitro* propagation of walnut (Payghamzadeh and Kazemitabar,2011)

Species Cultivars	Plant growth regulator								Study	Medium	Explant	References
	BAP	**Kn**	**TDZ**	**2,4-D**	**IBA**	**IAA**	**NAA**	**GA3**				
J. cinerea L	-	-	-	-	2.5 µM	-	-	-	Rooting	1/2 MS	Ms	Pijut (1997)
J. cinerea L	8.9 µM	-	-	-	-	-	-	-	Axillary bud culture	MS	Ns	Pijut 1997)
J. regia L	-	-	-	-	3 mg/l	-	-	-	Rooting of shoot	DKW	S	Heloir et al. (1996)
J. regia L	1 mg/l	-	-	-	-	-	-	-	Axillary shoot proliferation	MS	S	Heloir et al. (1996)
J. nigra L.	1-5 mg/l	-	-	-	-	-	-	-	Bud proliferation	MS or DKW	-	Sommers et al.(1982)
J. regia L.	1 mg/l	-	-	-	0.01 mg/l	-	-	-	Shoot multiplication	DKW, MS and WPM	St	Saadat and Hennerty (2002)
J. regia L	-	-	6.8 µM	-	-	-	1 µM	-	Adventitious shoot regeneration	DW (1/2 DKW +1/2 WPM)	L	Roschke and Pijut (2006)
J. regia L.	4.44 µM	-	-	-	0.005 µM	-	-	-	Shoot multiplication	DKW	Ge	Jay-Allemand et al.(1992)
J. regia L	1 mg/l	-	-	-	-	-	-	-	Shoot multiplication and rhizogenesis induction	MS	Eje	Penula et al. (1988)
J. regia L.	1 mg/l	2 mg/l	-	-	0.01 mg/l	-	-	-	Macro morphological and histological analyses.	Modified MS	Sb	Rodriguez et al.(1993)
J. regia L	1 mg/l	-	-		0.1 mg/l	0.05 mg/l	-	0.1 mg/l	Micro propagation	MS	Jns ,Ea	Revilla et al. (1989
J. regia L	4.4, 8.9 µM	-	-	-	-	-	-	-	Culturing nodal segement	Modified MS	Ns	Gruselle et al. (1987)
J. regia L.	0.4 µM	-	-	-	-	-	-	0.8 µM	Axillary bud elongation	MS	Ns	Chalupa (1981)
J. nigra L	-	-	-	5 µM	0.1 µM	-	-	-	Adventitious regeneration	WPM	-	Long et al. (1995)

4-Hardening and acclimatization of plants from *in vitro*

Acclimatization of successfully micropropagated plants is very important for commercial use. Plant losses experienced at this stage can cause all efforts to be wasted. Acclimatization of plants after micropropagation is actually a difficult step (Sanchez et al.2005). Ambient humidity and temperature are important at this stage. During acclimatization, plants are likely to be damaged by fungal diseases. In studies on this subject, it is important to use ventilated transparent containers and to perform the acclimatization process in fully controlled greenhouses. The survival probability of plants transferred to suitable humidity and temperature environments is quite high. As a matter of fact, in walnut research reports, it has been reported that more positive results are obtained from plants that are acclimatized in heat and humidity controlled greenhouses after *in vitro* (Stevens and Pijut, 2018).

Figure 3. Acclimatized walnut seedling

Result

The most important problem encountered in in vitro reproduction of walnuts is juglone, endogenous internal bacterial contamination and phenolic compounds that affect the materials used. The use of shoot tips, meristems and epicormic shoot tips at different physiological periods appear to be the best alternatives for in vitro propagation. There are many difficulties faced by the tissue culture industry in production. These; cost-effectiveness, automation, control and optimization of the microenvironment. A major challenge in walnut studies should be focused on genetic improvement of elite trees with desired treatments through transformation systems and identification with molecular markers, and these issues need to be studied in more detail in the future. Micropropagation of walnut with nodal segment culture has now become the most suitable method for clonal propagation of precious plant materials. Contrary to popular belief, walnuts can be easily reproduced vegetatively. Today, a large number of protocols have been established for the *in vitro* propagation of walnuts (Table 3). Thanks to micropropagation techniques, rootstock breeding studies for walnuts have gained momentum. In this way, mass rootstock production has begun. Currently, micropropagation companies have to deal with problems such as the effective cost of reproduction media, air conditioning and optimization. Recently, the use of agar-free liquid media has become widespread due to the cost of agar media.

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Part VI

Sustainable Farming Systems and Organic Walnut Growing

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Department of Horticulture, Usak, Turkey People and groups who saw that this increase in agricultural production, which was called the "Green Revolution" in the 1950s-60s, when chemical fertilizers, drugs, and chemical additives began to be used, did not bring a solution to the problem of hunger in the world in general, but on the contrary, deteriorated the natural balance, soil quality, and human health. They started various studies on the subject. Although the amount of production increased with these chemicals used over time, it killed beneficial organisms in the soil in the long term, causing a decrease in nutrient quality and soil fertility (Sinha and Herat, 2009). This situation necessitated the development of human and environment-friendly input and production systems in order to establish the natural balance lost as a result of these faulty practices in the ecological system.

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The first goal in organic farming is to preserve soil vitality and productivity. For this purpose, it is the addition of organic substances to the soil that ensures the continuity of vital activities without a natural-synthetic additive in the cycle of nature (Karacalar, 2008).

The amount and property of organic matter is one of important characteristics of soils. Organic matter plays an important role in many physical, chemical, and biological properties of soil. Organic matter improves the physical properties of heavy clay and light sandy soils, increases the water holding capacity and aeration of the soil. It also positively affects the chemical properties of the soil. It is a source of nutrients and N. It increases the cation exchange capacity, balances the soil pH, reduces the effects of toxic substances such as lime and excess fertilizer in the soil. It enables the plant to take insoluble plant nutrients into a soluble form and improves the living conditions of organisms in the soil (Karaman et al., 2007).

As a result of the intensive use of synthetic chemical inputs in traditional agriculture, chemical residues in products have increased. Organic farming includes the complete avoidance of the use of synthetic chemicals used in traditional agriculture that harm the environment (Altındişli, 1998).

Although most of the walnuts produced in our country are not named, they are organic. Walnut is one of the rare plants among the fruit species that did not use commercial fertilizers and pesticides in cultivation so far. This will provide great convenience in the transition to organic cultivation of walnuts.

The points to be considered in setting up a garden for organic walnut cultivation are similar to traditional (conventional) principles. The most important factor in establishing a garden for organic products is that the variety to be grown is suitable for the ecological conditions of the region (Özkan, 2005). It is a very important factor that fruit species and varieties are resistant to diseases and pests. While the size is an indicator of quality in conventional fruit growing, small fruit obtained without using any synthetic chemicals can be called high quality in organic fruit growing (Yalçınkaya, 2001). The most important issue to be considered in the walnut garden plant is the appropriate selection of the variety and pollinator variety. In addition, care should be taken to ensure that the grafted part of the seedlings to be used in the garden facility is an old and organic certified part. In the production of seedlings, non-organic or growth regulators that are not allowed to be used should not be used. Planting distances vary depending on the variety, rootstock, terrain, pruning, and harvesting. In addition, a planting distance that will minimize the risk of diseases and pests should be applied.

When weeds compete with trees in the intake of water and plant nutrients in organic fruit cultivation, weeds in the tree crown projection should be understood. Herbicides should never be used in the fight against weeds. Instead, mechanical methods, crop rotation, and mulch can be used. As a mechanical method, machine or manual cutting should be preferred (Anonymous, 2002).

Making green manure 1-2 years before planting will be an important gain, especially in terms of nitrogen source and soil improvement. Green fertilization after planting can be done by planting a legume plant such as vetch on the tree crown projection. The main food source is farm manure. According to the nutritional needs of the plant (between 1-5 tons per decare), well-burned farm manure should be given or organic farm manures prepared specially for their nutrient content should be used in recommended doses. It is appropriate to give fertilizers containing essential nutrients to the seedlings during the establishment of the garden. Then, other plant nutrients should be given according to soil and leaf analysis. One of the main reasons for the lack of yield and quality in walnut cultivation in our country is the plant nutrition problem. In order to determine this, soil analysis must be done and the appropriate fertilization program must be determined. In order to understand that the fertilization program of the plant is done correctly, leaf analysis should be done and according to this result, the missing plant nutrients should be supplemented. In addition to these, humic acid, seaweed, mycorrhiza, bacteria, etc. organic materials such as The most important issue that we will pay attention to in fertilization is that the products to be used are approved as organic fertilizers by the control and certification bodies (Altındişli, 1998).

Fertilization is the main factor affecting the yield and quality of trees. Walnut trees take many plant nutrients from the soil due to the development of roots, shoots, branches, leaves, buds, and fruits with the help of their roots. It is necessary to give these plant nutrients back to the soil.

The use of chemical fertilizers, against organic or natural fertilizers, is extremely common in the world and in our country. One of the main reasons for this is that it is easily available. Since chemical fertilizers reduce the vitality population in the soil, they make the soil increasingly self-dependent. In recent years, due to increasing fertilizer prices, producers have begun to be unable to obtain fertilizer, and they have turned the excrement of their animals into vermicompost, either directly or with the help of worms, and applied it to their gardens.

Organic farming is a sustainable farming system and a practice that includes the use of organic fertilizers (plant residues, manures of animal origin), based on specific production techniques such as disease and weed control. The main organic fertilizers used are;

Vermicompost

Vermicompost is a kind of natural organic fertilizer. Worms feed by carrying the plant residues that are thrown into the soil and start to rot, to the channels they open in the soil at night. As a result of their feeding, humus is formed from their feces and this humus plays an important role in increasing soil fertility. The common name of vermicompost in the world is vermicompost, and the cultivation of vermicompost is called vermicomposting (Şimşek, 2007).

Vermiculture is the general name for worm growing and vermicompost production. The main purpose of worm growing in vermiculture is to obtain vermicompost. But it is also part of the process to grow worms as a source of live and/or dried feed. Vermicompost is a product obtained as a result of composting various organic wastes (animal manure, straw, straw, household fruit, and vegetable waste, garden leaf waste, sawdust, waste paper, etc.) by some earthworms during their digestion and used as organic fertilizer and soil conditioner in the agricultural industry. Vermicompost is also called black gold. vermicompost; Due to its physical, chemical, and biological interactions in the soil and its slow release, it has become one of the most popular fertilizers of recent times (Türkmen, 2016).

Vermicompost is a very rich mixture in terms of plant nutrients and minerals, it gives root development to the plant with the fertilizer added to the soil. It increases productivity and improves the water holding capacity of the soil. Since it is an organic fertilizer, it regulates soil pH and soil structure. In addition, worm castings do not contain weed seeds (Anonymous, 2016).

sheep wool manure

Wool consists of keratin protein, which contains nitrogen, carbon, and sulfur, which is important in plant nutrition. It has been determined in the experiments that it has beneficial effects on the productivity of many plant species. Washed wool and its hydrolysates can be used as a valuable and environmentally friendly fertilizer (Tüfekçi and Olfaz, 2014).

seaweed fertilizer

Although seaweed is a soil improver, it is only suitable for local use due to its high transportation costs. The use of seaweed extracts as a foliar spray is quite common. In many crops, foliar sprays have increased yield. This is due to the micronutrients it contains along with the growth hormones it contains such as auxins. Seaweed extracts can be applied as liquid fertilizers as well as foliar sprays.

Seaweeds:

By providing strong root development, plants take more nutrients and water from the soil,

increase the green parts by accelerating the formation of chlorophyll in plants, therefore more carbohydrates, proteins, etc. They enable plants to be more resistant to diseases and pests, to make plants resistant to environmental stresses such as frost, drought, insufficient sun, excessive water, extreme heat, and extreme cold. It is a source of macro and micronutrients for plants. By putting the microelements, which cannot be taken by the plant in the soil, into chelate form, it ensures that the plant receives them at the highest rate and makes them balanced in the plant. It increases side branching and fruit set in fruit trees. It also reduces flower and fruit drop. It provides up to 30% increase in yield in plants. It increases the storage durability of the products. It inhibits the proliferation of viruses and reduces the damage of nematodes. By providing a balanced and long-term intake of macro and micronutrients from the soil, it increases the yield, improves the quality, and increases the market and export value (Blunden et al., 1992).

farm manure

Farm manure (barn manure) consists of the excrement of large and small cattle and the bedding laid under the animals in the barns. On the one hand, barn manure positively affects the structure of the soil, on the other hand, it has a positive effect on the amount of product by providing the necessary nutrients for the plants. These effects can be listed as follows: It increases the water holding capacity of the soil. It prevents water from flowing independently on the soil surface, evaporating and transporting arable land. It allows the soil to come to the pan easily. It makes the soil temperature suitable for plant growth. It has an effect on the pH of the soil. Barn manure has a positive effect on soil aeration due to its organic structure. On the other hand, carbon dioxide and organic acids formed as a result of the breakdown of barn manure in the soil make plant nutrients useful for plants. A large amount of microorganisms is given to the soil with barn manure. Thus, the rate of biological changes in the soil increases (Soyergin, 2003).

Humic acid

Humic acid is used as a fertilizer in plant nutrition. Humic acid, which is produced from lignite coal or leonardite by the extraction method, is impregnated with clay or compost and offered to the market as pellets or in the concentrated form under the name of organic fertilizer. Humine substances are important in plant nutrition, especially by increasing the rate of use of nitrogen and phosphorus fertilizers by breaking them down (Karaçal and Tüfenkçi, 2010).

It provides the ideal pH balance (5.5–7), the amount of organic matter (4–6%) in the root zone, regulates microbiological activity, balances the soil reaction of the environment by lowering the high pH value of salty and calcareous soils. It removes salt and lime from the root area by buffering. It has the feature of making the soil healthier, stronger, and a suitable environment for microorganism activities (Tunç, 2006).

Humic acids convert iron into an absorbable form and protect plants from chlorosis. It also helps the iron complex in the soil to be taken up by plants and the formation of chlorophyll in the leaves (Özbay, 2012).

Some studies on walnuts in the world and in Turkey are listed below with their results.

The study named “Investigation of the Effects of the Use of Composed Microbial Fertilizer on Yield and Quality Parameters in Walnut (cv. Chandler)” was carried out in the Demirci district of Manisa province between 2018-2019. (before the male flowers open, when the male flowers open and the fruits reach the size of hazelnuts) it was applied by spraying with the help of a leaf sprayer at a dose of 2 cc/L per tree, the highest results were obtained from the EM.A application in terms of fruit width, weight, and height in terms of the average of two years. In two applications, it was observed that the color of the fruits became darker but duller compared to the control group, in general, the amount of linoleic acid in the fruits was higher in the EM.5 application, followed by linoleic acid in the control group and oleic acid in the EM.A application, respectively, the highest yield was EM. In 5 applications (2.52 kg/tree), the lowest yield was determined in the control group (0.78 kg/tree). (Bilgin et al., 2020).

In a study conducted in the Kashmir region of India, four selections of walnut (SKAU/002 (S1), SKAU/008 (S2), SKAU/024 (S3), and SKAU/040 (S4)) were used in 6 different combinations of organic and inorganic fertilizers (T1). : all inorganic NPK fertilizer; T2: 50% manure, 25% vermicompost, 25% poultry manure; T3: 75% NPK fertilizer, 25% farm manure; T4: 75% NPK fertilizer, 25% vermicompost,; T5: % 75 NPK fertilizer, 25% poultry manure; T6: 75% NPK fertilizer, 25% evenly distributed worm, farm and poultry manure) the study was carried out, and looking at the results obtained, the best result was T4 application (tree height (16.12%), tree circumference (1.40%), tree canopy volume (37.85 m3), shoot elongation (0.81 m), fruit set (40.52%), fruit holding (58.21%), yield maximum increase. In addition, maximum fruit set (38.96%) and fruit holding (57.53%) were measured as higher results in S1 variety compared to other select varieties, S2 flood The highest yield (5.86 kg/da) was obtained from the cultivation compared to other cultivars (Wani et al., 2016).

In a study called "Organic Walnut Cultivation and Its Importance for Turkey", it was stated that cultural practices are very important for our country to be in the top places in terms of organic walnut production in the world, and this can be possible by combining traditional methods with new cultivation methods (Özkan, 2005).

In a study on walnut yield and soil quality in Kashmir, India, four selections [(SKAU/002 (S1), SKAU/008 (S2), SKAU/024 (S3), and SKAU/040 (S4)] and six fertilizer application in the first week of December [T1 (100% NPK fertilizer), T2 (50% farm manure, 25% vermicompost, 25% poultry manure), T3 (75% NPK fertilizer, 25% farm manure), T4 (75% NPK fertilizer, 25% vermicompost), T5 (75% NPK fertilizer 25% poultry manure) and T6 (75% NPK fertilizer, 25% fertilizer (1/3 farm manure + 1/3 vermicompost + 1/3 poultry manure) fertilizer)], the highest yield was obtained as a result of T4 application from S3 selection with 6.82 kg/tree, the highest nitrogen (338.59 kg/ha), phosphorus (20.80 kg/ha), calcium (1312.25 ppm), zinc (1.19 ppm), manganese (66.66 ppm), iron (55.95 ppm) and copper (2.74 ppm) contents were found as a result of T5 application, the highest potassium (259.27 kg/ha) and magnesium (268.86 ppm) T2 application was obtained (Wani et al., 2017).

Dr. In a study conducted in a 10-year-old walnut garden at YS Parmar University of Horticulture and Forestry, 13 different fertilizer applications were made, and according to the results obtained, 750 g nitrogen, 375 g phosphorus, 750 g potassium + 50 kg vermicompost, and 562.50 g nitrogen, It was observed that 281.25 g phosphorus, 562.50 g potassium + 68.50 kg vermicompost applications were effective on the leaf nutrient status of the trees (Bhattarai and Tomar, 2009).

In a study conducted in the Otovalo canton of Imbabura province of Ecuador, 13 different applications (T1= 25% Humus + 75% Rice husk, T2= 25% Humus + 75% Pomina, T3= 25% Humus + 75% Sand, T4=% 25 Humus + 75% Soil, T5= 50% Humus + 50% Rice husk, T6= 50% Humus+ 50% Pomina, T7= 50% Humus + 50% Sand, T8= 50% Humus + 50% Soil, T9= % A study on the germination time of walnut seeds with 75 Humus + 25% Rice hull, T10= 75% Humus + 25% Pomina, T11= 75% Humus + 25% Sand, T12= 75% Humus + 25% Soil, T13= Control) After the study, as a result of 50% Humus + 50% Pomina application, the highest germination and seedling number was reached with 99.38% (Cabascango, 2011).

In a three-year study with pecans, the combination of vermicompost and 3 different fertilizers (urea, potassium nitrate, Hydro complex (N

12.40%, P2O5 11.40%, K2O 17.70%, S 8%, Fe 0.20%) , Mn, 0.02%, Zn 0.02%, B 0.015)) with 4 replications, 4 different applications were made, in the applied applications (F1= 30 g/wood synthetic fertilizer, F2=60 g/wood synthetic fertilizer, 5 kg) /tree vermicompost and control group) soil properties and plant growth were examined, it was observed that there was no significant change in soil pH value, an increase in salinity was observed in the last two years of the study, provided with synthetic fertilization with a high stem diameter or organic improvement, the highest P in soil and C levels were obtained as a result of vermicompost application (Giuffré et al., 2017).

In a study conducted in the Kashmir region of India, the bio-efficacy of rhizobacteria isolated from walnuts grown in the Himalayas against five fungal pathogens in the future was investigated. As a result of the in vitro evaluation of rhizobacteria in terms of biocontrol effectiveness against 5 fungal pathogens, namely *Dematophora necatrix*, *Alternaria solani, Pythium aphanidermatum, Fusarium oxysporum* and *Phytophthora capsici*, it was determined that most of the bacteria belonged to the *Bacillus genus*. *D. necatrix*, *A. solani* of WI 90 (66%), WI 63 (55.6%), WI 62 (43.8%), WI 63 (45.5%) and WI 65 (49%) respectively. It was found to be antagonistic to *F. oxysporum*, *P. phanidermatum* and *P. capsici* and had maximum inhibition. The 12 most effective isolates were characterized morphochemically and molecularly and based on the 16S rDNA sequence, *Bacillus licheniformis* (Isolates WI90), *Bacillus subtilis* (Isolates WI63 and WI65), *Bacillus tequilensis* (Isolate WI62), *Bacillus cereus* (Isolate

WI36), *Micrococcus lute*, WI41, and WI80 isolates), *Micrococcus yunnanensis* (WI30 and WI60 isolates) and *Micrococcus* sp. (WI11 and WI91 isolates) (Sofi and Dar, 2018).

In a study titled "The Effect of Microbial Liquid Fertilizer on Rooting of Fig (Bursa Black), Reverse Mulberry, Red Mulberry, Walnut (Kaplan-86) Wood Cuttings", wood cuttings were taken from Fig, Reverse Mulberry, Red Mulberry, Walnut trees in two different periods during the winter resting season. , the cuttings were planted in plastic black pots containing perlite after being kept in 0, 25, 50, and 100 ml/L solutions of 10×104 (w/w) microbial organic liquid fertilizer overnight. While in mulberry, no rooting was observed in walnut, while increasing doses of organic microbial liquid fertilizer used in the experiment had a negative effect on rooting, it was observed that the optimal dose of 50 ml/L had a positive effect on root length, root diameter, shoot length and shoot diameter in cuttings planted in autumn (Dalkılıç et al., 2019).

The mineral nutrition status of walnut orchards in Uşak was investigated by soil and leaf analyzes. and copper; In the leaf samples, additional nitrogen and boron mineral analyzes were made to these elements, according to the analysis results, 39% of the soils of the gardens are loamy, 57% are clay loam, salinity is between normal values, approximately 61% of the soils have lime problems, pH value It was determined that it is neutral or slightly alkaline, mostly in the middle class in terms of organic matter content, 69% of the orchards are poor in phosphorus, 78% in magnesium, 96% in zinc and 98% in potassium; According to the results of leaf analysis, it was observed that 29% of walnut orchards were deficient in phosphorus, 76% in potassium, 80% in magnesium, 78% in iron, and 94% in zinc and copper (Yıldız and Uygur, 2016).

In a study on the “Western” variety of pecan nut, anaerobically digested biosolids were applied to pecan trees for three years, and it was observed that the fruit shoots increased by 16% and the yield per tree increased by 11.3% as an average of three years, and heavy metal concentrations (As, Cd, Cr, Hg, Ni and Pb) were found to be decreased, also Salmonella spp. And Escherichia coli was not found (Tarango-Rivero et al., 2011).

In a study on pecans, it was observed that one of the most important factors in the development of walnuts is nutrition, and some of the direct-acting elements for the best development of the fruit are nitrogen, phosphorus, potassium, zinc, and boron (Acevedo-Barrera et al., 2013).

In order to make the rock phosphate in the soil useful, two different nitrogen-fixing bacteria (*Pseudomonas chlororaphis* and *Bacillus megaterium*) and two different phosphate-solving bacteria (*Arthrobacter pascens* and *Burkholderia cepacia*) were inoculated into the soil, the highest phosphorus solubility was obtained from the mixture of *Pseudomonas chlororaphis* and *Arthrobacter pascens* bacteria. , a strong correlation was found between total organic acid production and phosphorus solubility as well as pH and soluble *phosphorus correlation*. In addition, the concomitant use of *Bacillus megaterium* and *Arthrobacter pascens* was unsuccessful, and it was concluded that co-vaccination of *Pseudomonas chlororaphis* and *Arthrobacter pascens* bacteria would be a good alternative to fertilization (Yu et al., 2012).

In a study conducted in California, “Paradox”, which is widely used as a walnut rootstock, is sensitive to *Agrobacterium tumefaciens,* and the use of methyl bromide and Telone-C35 (1,3-dichloropropene and chloropicrin) is common in that region against this important problem. It was stated that these applications significantly changed the microbial activity in the soil over time. It was assumed that the increase in microbial activity and diversity in fumigated soil would cause competition against *A. tumefaciens* and decrease its density, the study was initiated by commercially procuring three soil types (vermicompost and two different fermented microbial fertilizers), the study continued for 4 weeks, two Although different microbial fertilizers did not have any effect, it was determined that vermicompost reduced the *A. tumefaciens* population, and the reason for this was biotic (Strauss et al., 2015).

In a study, a trial was set up in walnut plant using 3 synthetic and 3 natural and microbial inputs, N=0 per cm of trunk diameter; 16; 80; 160 and 320 g; P2O5=0; 7; 35; 70 and 140 g; K2O 0; 7.5; 37.5; 75 and 150 g; compost 0; 250; 1250; 2500 and 5000 g; vermicompost 0; one hundred; 500; 1000 and 2000 g; mycorrhiza 0; 3.81; 19.05; It was applied as 38.00 and 76.20 g, and as a result of the study, 226 kg/ha nitrogen, 121 kg/ha P2O5, 94 kg/ha K2O, 3111 for 1.94 t/ha, 149 fruit/kg and 59% edible walnut production kg/ha compost, 1905 kg/ha vermicompost and 33.02 g/cm mycorrhiza were used. It was also observed that the required doses for production were 30% nitrogen and organic improvers, 50% phosphorus and potassium, and mycorrhiza contributed 95% to fruit quality; it was also determined that fertilizer costs constitute 40.8% of the total expenses (Flores-Cordova et al., 2018).

In order to control *A. tumefaciens* and other soil and seed-borne phytopathogens in walnut nurseries, anaerobic soil disinfection was applied considering it could be an alternative option compared to fumigation, and after this application, its potential was examined together with vermicompost. In this study, 20 metric tons/ha of soil was covered with rice bran, covered with TIF for 6 weeks after watering for 24 hours. To examine the effects of anaerobic soil disinfection efficiency and vermicompost application after anaerobic soil disinfection on disease incidence, Paradox walnut rootstock seeds were dipped into A. tumefaciens inoculum before planting and planted in such a way that anaerobic soil disinfection was applied to the place where anaerobic soil disinfection was applied. The amount of A. tumefaciens was found to be significantly less than the areas without vermicompost compared to the control plots planted with walnut seeds and vermicompost (Sarah et al., 2014).

In a study, walnut plants were grown in 10-liter pots in soil mixed with three different organic materials (vineyard compost, liquid vermicompost, fulvic acid), solid fertilizers were added to the soil at the first planting and liquid ones were added to the soil every week for 16 weeks, plant growth parameters were determined during the season. During the incubation and pot experiments, Zn extractability was increased by C ratio ($p<0.05$), and a significant positive correlation was found between water-soluble C and DTPA-Zn, Zn extractability was highly affected by the Zn and C source in the pot experiment ($p<0.05$), it was observed that humic substances were more effective in terms of Zn extractability compared to the control, vermicompost and fulvic acid provided the highest Zn extraction, the highest number of shoots and leaves with 24 kg Zn/ha-C equivalent application and the highest obtained with 12 kg Zn/ha+FA at low C ratio (Molina et al., 2015).

In this study to examine the effect of liquid vermicompost on the survival and growth of walnut seedlings, it was found that the use of bio-preparation had a positive effect for this culture, plant survival rate after diversion was 28% and growth parameters (average number of branches per plant, number of leaves). , average branch growth rate per plant) by 11.5% – 54.1% (Faleyev et al., 2019).

In a study on plant growth in 1+0 aged Humic acid and biohorm walnut seedlings of vermicompost, a product of Biohumus®, grown in Bartın-Turkey, seedlings were placed in black seedling bags in loem soil, a sandy soil, and humic acid (0, 2, 4) ml/L and biohorm (0, 3, 6) ml/L solutions containing different concentrations were applied by spraying on the leaves at 15-day intervals on three dates, respectively, 20 April, 5 May and 20 May, data were collected in September and There was a significant increase in shoot number, leaf number, shoot diameter, shoot dry weight (gm), root number and leaf surface area (cm^2) in seedlings treated with 4 ml/L humic acid solution and 6 ml/L biohorm solution compared to the control group. It was observed that the highest chlorophyll pigment was observed in the seedlings applied 3 ml/L biohorm (Taha, 2017).

In the study carried out at an average altitude of 900 m in the Yeşilyayla neighborhood of Kula district of Manisa province in 2019, the domestic walnut varieties Bilecik, Kaman-1, and Şebin were found to be treated with Solid Vermicompost, Liquid Vermicompost, Solid Sheep Wool Pellet, Liquid Sheep Wool Pellet, Solid Seaweed and Sebin. Some fruit quality characteristics of liquid seaweed fertilizers applied from soil were investigated. 20 fruits were selected from each application. As a result of the study, the fruits with the highest fruit weight were obtained as a result of the application of Solid Worm Fertilizer in Sebin variety with an average of 11.85 g, the fruits with the highest internal weight were found in the Kaman-1 variety with 7.10 g. As a result of the application of Liquid Sheep Wool Pellet, the fruit internal yield The highest amount of fruits was observed in the Solid Seaweed application of Kaman-1 variety with 62.04%, the highest protein ratio was found in Kaman-1 variety with Liquid Worm Fertilizer application with 30.54%, and the highest Phenolic Compound amount was 1145 ppm in Bilecik. It was observed that it was obtained as a result of the application of liquid Sheep Wool

Pellet. In addition, it was observed in this study that the organic fertilizers that most affect fruit quality characteristics are Solid Vermicompost, Liquid Vermicompost, and Liquid Seaweed (Çolak and Karaca 2021).

Conclusion

Turkey is a country suitable for organic production with many factors such as its geographical conditions, climate, product variety, and the high number of people working in agriculture, and the demand for organic agriculture is increasing day by day. Organic (ecological) agriculture includes the complete avoidance of the use of synthetic chemicals used in traditional agriculture that harm the environment. The goal in organic fruit cultivation is to create the best conditions for human and environmental health in all stages from the starting material of production to the delivery of the fruit to the consumer, without the producer incurring economic loss compared to other agricultural systems. The organic farming system has an important place in agricultural practices. It is necessary to carry out the necessary studies for the spread of this agricultural practice. With the increase in support and marketing opportunities, the demand for this system will increase even more. The majority of walnuts produced in our country are organic, although they are not named. It is one of the rare fruit species that does not use synthetic fertilizers or pesticides in cultivation so far. This situation provides great convenience in the transition to organic cultivation of walnuts. The most important factor in creating a walnut garden is that the variety to be grown is suitable for the ecological conditions of the region and is not damaged, especially from late spring frosts. The grafted part of the seedlings to be used in the garden facility must be an old and organic certified part. In organic farming, issues such as fertilization and fight against pests, garden location, variety selection, seedling quality, training system and pruning technique, irrigation, tillage, and harvest are important cultural processes.

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Part VII

Industrial Use of Walnut

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Introduction

Walnut (*Juglans regia* L.) is one of the members of Juglandaceae family. *Juglans* genus has 20 species of many with economic significance. These species are distributed in the temperate and subtropical climate zone of the world. Among these species, the most economically important species is *Juglans regia* L. Walnut (*Juglans regia* L.) is one of the hard-shelled fruit taxa that is deciduous in winter (Akyüz and Serdar, 2017; (Doğu et al., 2001); (Dönmez and Güler, 2015).

Today, walnut is a type of fruit that is grown in many countries of the world due to valuable fruits (Akyüz and Serdar, 2017); (Dönmez and Güler, 2015); (Mitrović et al., 2007). Walnut is a native plant of a

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wide region from the Carpathian mountains to Turkey, Iraq, Iran, Afghanistan, Southern Russia, India, Manchuria, and Korea (Sandal et al., 2008).

In 2019, 3.7 million tons of shelled walnuts are produced in the world. China ranks first in the cultivation area and production amount of walnut in shell followed by America and Iran (Table 1). Turkey as the fourth proceeding country in world walnut production is both an importer and an exporter of walnut. The countries with the highest income from walnuts worldwide are the USA and France. However, walnut production in Turkey is increasing every year (FAOSTAT, 2021).

In Turkey, walnut cultivation is carried out in almost every province except for a few ones. Providing technical support from production to processing, organization, and marketing of fruits are factors affecting the economical value in walnut production both in domestic and foreign markets (Aksoy and Kaymak, 2021); (Akyüz and Serdar, 2017); (Dokuzlu, 2020); (FAOSTAT, 2021).

Table 1. Walnut (with Shell) Production in 2019 for Countries with a Descending Order (Top 20 Countries with Tonnes) (FAOSTAT, 2021)

	Area	Value
1	China	2521504
2	United States of America	592390
3	Iran (Islamic Republic of)	321074
4	Turkey	225000
5	Mexico	171368
6	Ukraine	125850

7	Chile	122950
8	Uzbekistan	50660
9	Romania	49580
10	France	34950
11	Greece	31040
12	Egypt	24013
13	Argentina	18488
14	Belarus	18431
15	Republic of Moldova	17706
16	Spain	17540
17	Pakistan	14862
18	Afghanistan	14690
19	Azerbaijan	11581
20	Italy	10800

FAOSTAT (Access date: 2nd Descember, 2021)

Walnut cultivation is common in Anatolia due to its place in the diet of people living in Mediterranean countries and the long storage period of walnut (Amaral et al., 2003).

The cultural history of the walnut is old and it is a species with high genetic diversity. Moreover, the ability of walnut to adapt to different environmental conditions makes its cultivation worldwide. It is a species that has been researched a lot due to its nutritional content, industrial use, and economic return. The global popularity of walnut fruit is due to its high nutritional, health, and sensory properties (Bayazit et al., 2016); (Martínez, 2010).

There are numerous studies on the chemicals, phytochemical and biochemical properties of walnut fruits. Studies on the contribution of walnut fruits to human health and nutrition cannot be ignored. Walnut kernels have a high amount of vitamins, minerals (K, P, Mg, Ca, S, Cu, P, etc.), fat, protein, and polyphenols (Guney et al., 2021); (Okatan et al., 2021) (Kafkas et al., 2020); (Rusu et al., 2018); (Simsek, 2016); (Panth et al., 2016). Protein, vitamins, and minerals increase the biological and nutritional value (Ebrahimi, 2018); (Yiğit, 2005).

Walnuts are a rich source of vitamin E. Walnut kernel also contains vitamins A, C, B6, thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), and folic acid (B9). Moreover, it has been reported that walnuts are a good source of magnesium, calcium, manganese, potassium, sodium, and copper (Şen and Karadeniz, 2015); (Yiğit et al., 2005), flavonoids and phenolic acids Arcan et al., 2021);(Ebrahimi et al., 2018); (Martínez, 2010); (Trandafir et al., 2016).

Walnut has a wide range of uses in preventing diseases and supporting plant-based drugs used in diseases treatment. Due to this feature, walnuts are considered as a 'functional food'. One of the most important components that make the walnut functional is the oil it contains (Bakkalbaşı et al., 2010); (Yiğit et al., 2005). Walnut kernels usually contain about 60% oil, but this can range from 52% to 70% depending on the variety, geographic location, and irrigation rate (Oliveira, 2002); (Moser, 2012); (Yiğit et al., 2005).

In terms of nutrition, the composition of the oil is more important than the amount of oil. While some of the nuts (E.g. almonds) are rich in monounsaturated fatty acids (MUFA), walnuts contain polyunsaturated fatty acids (PUFA) at a higher rate. One of the most important features of walnut oil is its high amount of linoleic acid (Sabaté and Hook, 2020); (Yiğit et al., 2005). There is a lot of literature reporting the total SFA (Saturated Fatty Acid) (4-7.86%; 8.8%), total USFA (Unsaturated Fatty Acid), total PUFA (84%; 72.84%; 71.43 - 62.73%) and total MUFA (1%; 28.53 - 22.17%;) values of walnuts (Copolovici et al., 2017); (Simsek, 2016); (Tsamouris, 2002). About 80% of unsaturated fatty acids are linoleic acid (omega-6), and 20% are α-linolenic (omega-3) acids. The ratio of omega-6:omega-3 has been determined as 5.06 which could help in a human healthy diet (Copolovici et al., 2017); (Şahin, 2005); (Willett et al., 1995).

Walnut application in the pharmaceutical industry:

Walnut has a long history in folk medicine, especially in ancient China. Although the use of chemical and synthetic drugs became more common in the last half-century, their harmful side effects quickly led to a re-emergence of natural base drugs. Plant-derived drugs have always been one of the most effective methods throughout history.

The studies proved that walnut has a protective effect against cardiovascular diseases and raises good cholesterol (HDL) in the blood (Şahin et al., 2005); (Savage et al., 1999); (Wu et al., 2014);

(Zibaeenezhad et al., 2005); (Zibaeenezhad et al., 2003). Moreover, it has been reported that walnut has the ability to delay or reduce the development of very strong diseases such as Parkinson's and Alzheimer's. Walnut consumption can increase the level of melatonin in the blood. Walnut has a positive effect on the intelligence development of children. Due to its fiber content, it plays a beneficial role in the human digestive system. It can contribute to the elimination of disorders such as sleep disorders (Şimşek and Gülsoy, 2016); (Serrano, 2005); (Reiter et al., 2005).

Modern pharmacy studies reported that walnut regulate blood circulation, shows antiallergic properties, regulate liver functions, and promotes protein synthesis. For the past years, it has been reported that people have been using walnuts as a diuretic, stone remover, anti-vomiting during pregnancy, weight gain, and calming (Kim and Choi, 2021); (Yiğit et al., 2005); (Milind and Deepa, 2011).

In addition to the inner part of the walnut fruit, it is known that the walnut leaf and shell have been used in alternative medicine for many years. Walnut leaf pharmacologically has vasoconstrictor, hypoglycemia, antifungal, wart removal, diarrhea suppressant, and skin cleansing effects. It has also been reported to be vascular protective and tumor inhibitory (Girzu, 1998). Walnut leaves are used in the treatment of skin inflammations and ulcers, as well as in alternative and modern medicine treatments thanks to their antiseptic properties. It has also been reported that walnut leaves are used externally in skin inflammation, hand and foot sweating, acne and wounds, eczema, herpes, bee stings (Almeida, 2008); (Yiğit et al., 2005). In addition, it has been reported that the porridge obtained from walnut leaves contributes to the healing of acne, wounds, and swollen glands (Şimşek and Gülsoy, 2016). Walnut fruit shell also has therapeutic properties for various diseases (Yiğit et al., 2005).

Walnut application in dying and food industry:

In addition to walnut application in the pharmaceutical industry, different parts of walnut also are used in other various industries. Walnut leaves, husk, or tree bark are among the important natural dyestuffs. Thus, before the development of the dye industry, it has been used for centuries to obtain different brown tones, especially for dyeing wool yarn (Şahin, 2005); (Arifeen et al., 2021).

It has been stated that the outer shells of walnuts are used as a flavoring agent in the production of some traditional liqueurs (Stampar, 2006). Moreover, walnut shells have the potential to be used in different areas of the food industry (Doğan et al., 2014).

In recent years, wastes generated as a result of rapid industrialization constitute a major problem for living life and the environment. The application of appropriate adsorbents in the removal of colored and colorless organic pollutants in industrial wastewater draws attention as a significant usage of the adsorption process. In a study, it was aimed to obtain a cheaper, more abundant new natural adsorbent substance that can be an alternative to activated carbon, which is widely used in heavy metal purification. For this purpose, walnut wood chips were used as adsorbents. As a result of this study, it has been reported that walnut wood sawdust can be an alternative adsorbent to activated carbon (Çakır et al., 2013); (Kaya et al., 2011). In addition, walnut wood sawdust can be a suitable adsorbent in chrome removal (Çakır et al., 2013).

Walnut tree has very valuable wood. For this reason, it is used in furniture production, parquet production, sports, and musical instruments, carving, dowry chest production. In addition, it is used in the arms industry for the manufacture of some parts of rifles and pistols (Dönmez and Güler, 2015).

Walnut application Energy industry

Walnut pruning and shell materials can be used in obtaining electricity and biofuel (Dönmez and Güler, 2015); (Halil, 2005); (Moser, 2012); (Folaranmi et al., 2016); (Ardebili et al., 2011).

The high amount of oil content makes the walnut to be considered a good source for the biodiesel industry. In parallel with the increase in the world population and developing technology, the energy demand is constantly increasing. The fact that fossil energy resources are limited and will be depleted in the near future has revealed the necessity of evaluating alternative energy resources today. Factors such as the depletion of industrial oils, the increase in their prices, the damage they cause to the environment, and the increase in environmental awareness have increased the interest in renewable energy sources such as biodiesels. Biodiesel is an alternative fuel for diesel engines which can be produced from renewable resources such as vegetable and animal oils (Knothe, 2010); (Borugadda and Goud, 2012); (Mekhilef et al., 2011); (Hosseini and Wahid, 2012); (Halil, 2005); (Van Gerpen, 2005); (Demirbas and Karslioglu, 2007); (Krawczyk, 1996). Biodiesels are non-toxic, renewable, and biodegradable with low CO2 and NOx emission profiles and, therefore, are environmentally useful (Krawczyk, 1996); (Hossain et al., 2018); (Folaranmi et al., 2016). Although biodiesel and diesel fuel have similar physical and chemical properties, biodiesel fuel properties are better than petrodiesel fuel (Balaji and Cheralathan, 2013); (Demirbas, 2007); (Canakci, 2007); (Sharma, 2005). Cetane number which reduces the ignition delay is a significant criterion in evaluating the quality of biodiesel fuel. Biodiesel has a higher cetane number (Sivaramakrishnan and Ravikumar, 2012); (Shahid and Jamal, 2011). Biodiesel emits fewer emissions such as CO_2 (carbon dioxide), CO (carbon monoxide), SO_2 (Sulfur dioxide), PM (particulate matter), and HC (hydrocarbon) compared to diesel (Gupta and Agarwal, 2015); (Mofijur et al., 2016); (Mofijur et al., 2015); (Agarwal et al., 2018); (Macor and Pavanello, 2009); (Agarwal et al., 2016). Biodiesel is safe to process and transport and does not need to be refined (Demirbas, 2007).

Appropriate raw material selection is very important for biodiesel production. More than 350 oil-bearing crops have been identified as potential sources for biodiesel production (Atabani et al., 2012); (Balat and Balat, 2008); (Mofijur et al., 2012); (Silitonga et al., 2011).

The most common edible oil sources are soybean, palm, sunflower, cottonseed, rapeseed, peanut, castor, jojoba, corn, olive, coconut (Meneghetti, 2007); (Obadiah et al., 2012); (Pimentel and Patzek, 2005); (Sandouqa and Al-Hamamre, 2019).); (Veljković, et al., 2018); (Yee et al., 2009); (Venkatesan and Sivamani, 2019).

Walnut seed oil is among the edible oils that can be processed as biodiesel (Folaranmi et al., 2016). Properties of walnut oil such as density at 25°C, acid value, saponification value, iodine value, peroxide value, ester value, and kinematic viscosity are as in Table 4 (Folaranmi et al., 2016) and walnut biodiesel properties are given in Table 5 **(**Aydoğan et al., 2020).

Table 4: Physicochemical Properties of Walnut (*juglans regia* L) Oil (Folaranmi et al., 2016).

Parameters Walnut	(*Juglans regia* L) oil
Relative Density (Kg/l)	0.873
Flash Point (°C)	156
Iodine Value (Wij)	75.05
Acid Value (Mg KOH/g)	0.846
Saponification Value (Mg KOH/g)	308
Peroxide Value	56
Free Fatty Acid (Mg KOH/g)	0.423
Ester Value	307.577
Kinematic viscosity (mm^2/s)	34.5

Table 5 . Physical Properties of Walnut Biodiesel and Eurodiesel (Aydoğan et al., 2020).

Specifications	Walnut Biodiesel	Eurodiesel
Density (gr/m3)	0,893	0,827
Viscosity (mm2/s)	5,23	2,82
CFPN (˚C)	-3	-20
Calorie Value (MJ/kg)	40,6	47,5
pH	5.5	6

Biodiesel produced from walnut oil and its mixtures with euro diesel fuel can be considered as an alternative fuel for diesel engines (Aydoğan et al., 2020).

To summarize, walnuts can be used primarily in the food sector, but also in the chemical, pharmaceutical, cosmetic, furniture, paint, and fuel sectors (Bakkalbasi et al., 2015); (Beiki et al., 2018); (Grosu et al., 2012); (Qin et al., 2005); (Saxenaa, 2009).

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Part VIII

Diseases in Walnut Orchard

Dr. Kamil Sarpkaya[1]

Walnut Blight:

The causal agent of the disease is the bacteria, *Xanthomonas campestris* pv. *juglandis* (Pierce (Dye), which is previously named *Bacillus juglandis, Bacterium juglandis, Phytomonas juglandis, Xanthomonas juglandis*.

Xanthomonas campestris pv. *juglandis* is aerobic, gram-negative, non-spore-forming, rod-shaped bacteria.

Walnut blight is regarded as an important disease from the economic point of view, and it can be easily detected where walnut is grown in the World.

Causal agent bacteria, mainly overwinter in buds and catkins of walnut and dormant buds, can lead to an infestation of internal bud parts and developing fruits (Miller and Bollen, 1946; Mulrean and Schroth, 1982). Rain splash also causes suspend of bacteria in the air, and it might be the main factor for carrying inoculum to developing

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fruits by winds (Stall et al., 1993). It is also claimed that infected pollens may also transport the inoculum, but it has not been proved yet. Walnut blight makes infection on fruits (mainly), catkins, shoots, and leaves (Figure 1).

Figure 1. Initial Symptoms of Walnut Blight on Catkins (a) Developing Fruits (b) and Shoots (c)

Initial symptoms of the disease are seen on catkins with several black spots. Infection occurs at the apical or blossom end initially and lesions of bacteria increase in the side or apical end of developing fruits with black and water-soaked bands surrounding lesions (Figure 2). According to the severity of the disease, lesions size enlarges in infected areas. The band may disappear thoroughly when the pathogen stops colonization in unaffected tissues. Infection may develop in whole or part of the kernel, as well. In the case of favorable conditions for the occurrence of this disease in spring, fruits may drop prematurely, and yield significantly declines.

Figure 2. Apical Lesions and Developing Water-Soaked Bands on Fruit (Photo Credit: Florent Trouillas)

As the plant aged, the formed lesions are suppressed and bacteria form ooze in the lesions together with the decomposed cell materials in higher humidity levels. Lesions on fruit may range from a few mm to over half the size of the outer part of the fruit. Early infections develop with blackening or rotting in the hull before the hardening of the shell. Shrivel of kernel causes premature fruit dropping. Lesions develop on outer parts of the fruits in later infections and such fruits remain on the tree until harvesting time. The quality of the nut is also affected because of the possible adherence of the infected hull to the hard shell, resulting in discoloration and stain after dehulling (Lang and Evans, 2010). In favorable conditions for disease, lesions may develop in the kernel and cause the rotting of kernels (Figure 3).

Figure 3. Progression of Walnut Blight Severity in Kernel (Left to Right) (Photos: https://shop.agriad.ir)

Control of Disease: Using healthy plants for launching an orchard is essential. Distance between the trees is also important for reducing the risk of disease incidence. Water level should be kept balanced in irrigation, and sprinkler irrigation should be avoided especially in flowering time. Cultural practices, such as balanced fertilization, weed control, ought to be implemented properly.

Due to the uncertainty of epidemiological aspects, management strategies of disease are not well defined (Moragrega and Ozaktan, 2010). Although a spray prediction model has been improved in recent years, the control of disease is not guaranteed because of the effects of several factors for disease development. Walnut blight is not well controlled in the areas where the climatic condition is favorable. However, protective treatments can be used according to rainfall duration in spring at ten days interval starting bud break to fruit set of walnut orchards, having a disease history of walnut blight (Ninot et al., 2002). It is highly recommended for full coverage spraying. Mummified fruits should be removed for reducing inoculum.

Walnut Anthracnose

Walnut anthracnose, caused by *Ophiognomonia (=Gnomonia) leptostyla* (Fr.) Sogonov, has been reported as the most important and common fungal disease of black walnut (*Juglans nigra* L.) or English walnut (*J. regia* L.) throughout all walnut production regions in the World (Belisario, 2002; Belisario *et al.*, 2008; Hassan and Ahmad, 2017).

The causal agent is well described by many researchers. Fungus produces acervuli in early spring with black specks on abaxial of leaves. Conidiophores are short, elliptical, hyaline, and one-celled packed together in a small layer bearing conidia at the tips. The shape of conidia can be straight, ovoid, falcate, or be rounded at one end, while the other end is pointed. The characteristic of fungi is possessing one septum and two globular unequal cells with the size of 15- 26 × 2-5 μm. Immersed perithecia are composed of fallen leaves in brown color at the end of the season, while the beak protrudes considerably onto the leaf surface (Hassan and Ahmad, 2017).

O. leptostyla makes infections by ascospores during May, and it causes initial lesions on leaves (Figure 4). Seconder infections are made by conidia form of fungus, and the number of lesions increases in early summer. Furthermore, the development of disease reaches a maximum level in late July and early August. Defoliation might be seen on previously infected leaflets (Kessler, 1984). According to Rosnev and Naidenov (1986), the fungus is favored of the temperature of 15-30°C, frequent precipitation, and humidity over 65 percent. The severity of infection is even more on older leaves and 10 times more frequent with adaxial inoculation (Matteoni and Neely, 1977).

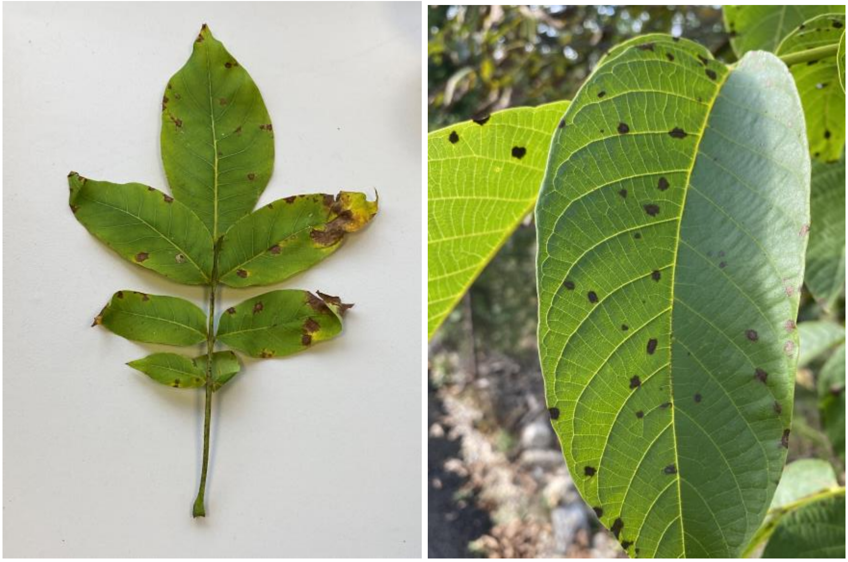

Figure 4. Initial lesions of Anthracnose on Walnut

Decreasing in nut yield due to anthracnose varies from variety to variety. Based on the report, there is a relationship between the size of the spots on the leaf and fruit pericarp and size, nut development, and yield. Because of spots forming after infections, metabolic processes disturbance and changes in biochemical indices in leaves are expected (Shirnina and Kotljarova, 2000). As a result, the fruits may be smaller than their normal size, poorly filled, of low quality with darkened kernels (Black and Neely, 1978; Zamani et al. 2011), as well as low yielding (Pinter et al. 2001) (Figure 5)

Figure 5: Effect of Various Severity Levels of Anthrachnose on Walnut Fruits (http://www.baghbantak.com/index.php/nuts-trees/walnut/walnut-anthracnose)

The initial symptoms of the disease can be seen on current year leaves, fruits, and twigs, while shoots might be rarely infected. At the first stages of the disease, infected areas on leaves are seen as brown to black colors with regular or irregular circular spots. Later on, large necrotic areas are composed by enlarging and coalescing of these spots. Eventually, these infected leaves change to yellow and early defoliation is seen on the trees according to the severity of the disease (Kalkism, 2012) (Figure 6)

Figure 6. Characteristics of Lesions and Early Defoliation on Trees

Control of Disease: Management of disease, based on both cultural practices and chemical control, was broadly studied by various researchers in different countries. Fallen leaves need to be buried to a depth of 10-15 cm, on the other hand, infected twigs should be pruned well, and eradicated, and adequate nitrogen fertilizer will be helpful to control the disease in orchards (Pscheidt and Ocamb, 2014). Moreover, some resistant or less susceptible varieties are recommended to set an orchard in the area where the climatic conditions are favorable for disease.

To ensure the efficiency of chemical control of the disease, diagnosis the proper and most effective time of chemical application in the initial stages of the disease, aerial spore dispersing, is of high importance. Application of Bordeaux mixture in late winter and copper compounds in early spring, just before sprouting, is far effective to control walnut anthracnose (Zamani et al. 2011). Furthermore, new generations of systemic fungicides have been widely used in different countries in recent years.

Thousand Cankers Disease

Thousand cankers disease is actually caused by a combination effect of canker-producing fungus (*Geosmithia morbida*), which is inserted into trees by an insect vector named as the walnut twig beetle (*Pityophthorus juglandis*). Those organisms cause a decline in branches of trees that is resulted from the cumulative injury.

More likely, two organisms (fungus, twig beetle) are steadily found together to indicate the occurrence of thousand cankers disease. A second fungus (*Fusarium solani*) is also associated with trunk cankers in advanced stages of Thousand Canker Disease (Cranshaw and Tisserat, 2012). (Figure 7).

Figure 7. Damage of Walnut Twig Beetle and Developing Canker Tissues on Walnut Twigs (Photo: http://www.invadingspecies.com/invaders/forest/thousand-cankers-disease/#bwg23/88)

Defoliation on the crown part of the tree is a common characteristic of disease which is followed by yellowing or sudden leaf wilting.

Generally, such symptoms on trees cause dieback or death of all trees in subsequent years because of faster progress of the disease after infestation (Figure 8). Development of the disease varies according to plant vigor, host cultivars or species, climatic conditions, and natural controls of the walnut twig beetle.

Figure 8. Dieback of Walnut Trees by Thousand Cankers Disease (Photos:https://www.udel.edu/academics/colleges/canr/cooperative-extension/fact-sheets/thousand-cankers-walnut/)

Based on symptoms, the disease is well identified in mid-summer. Furthermore, research has shown resides in the vascular tissue of trees, and until a change in climatic conditions (such as water stress) activates it, stays hidden. Therefore, in stress conditions, such as drought or high temperature, the disease progress can be faster. The severity of the disease in both cases depends on the sensitivity of the host, the length of the incubation period, and the presence of the wounds.

Control of disease: It is recommended neither apply insecticide to control walnut twig beetle nor fungicide. Instead of chemical control, some cultural practices, such as sanitation of infested materials, adequate fertilization, and proper irrigation in orchards, can be implemented. In the condition of less than 50% of the alive crown, main branches and scaffolds should be removed. To avoid the onset activity of walnut twig beetle, plant materials after any practices like pruning should be removed from orchards.

Shallow bark canker

Shallow bark canker is caused by a bacterium belonging to genus *Brenneria* (called *Erwinia* previously), and subsequently named as *B. nigrifluens* (Hauben et al., 1998). The disease is characterized by the symptoms demonstrated blacken spots or areas on the trunk or main branches of trees. Areas with a marginal water-immersed lesion on bark with vertical oozing represent recently infected areas, which is followed by dried spots with a thick resin (Figure 9). When the surface of lesions is scratched, forming of dying tissues can be detected under the bark. General symptoms of the disease appear as weakness, deterioration, and dieback of the scaffolds. Initial dieback starts unilaterally and finally the whole tree dies. Bacteria overwinter inside the canker or its secretions and in spring, it goes out with the exudates. Then, spread out to other trees through wind and rain, and penetrates inside especially through wounds on trees. Shallow bark canker remains active for many years and are very active, especially in summer, but become inactive during autumn and winter.

Figure 9. Necrotic Lesions of Shallow Canker (left) and Vertical Oozing from Infected Areas (Photos: K. Keshavarzi and H. Karimipourfard, 2021)

The disease occurs due to the weakening of the tree resulting from environmental stresses such as soil compaction, nutritional deficiencies, over-irrigation, crown, and root rot. Even recovering trees will be still prone to show the symptoms if they are exposed to these stresses again.

Control of Disease: The disease develops rapidly in poor and dry soils. Proper nutrition and regular irrigation as well as the use of resistant cultivars are the most important strategies for managing this disease. Tissues involved with the disease can be removed, then treatment with copper compounds is helpful.

Crown Gall

Crown gall is caused by *Agrobacterium tumefaciens* which is a rod-shaped and gram-negative bacterium, and it often appears as tumors or galls of various sizes on roots, crown, at or beneath the soil surface. Galls rarely occur on the upper parts of trees or the trunk. Wounds on plants and also the inoculum attached to the injured areas are general infection points, so some operations resulting in an injury either in nurseries or orchards drastically increase the risk of disease. After penetration of bacteria, the infected parenchymatous and vascular tissue grow rapidly with a bright color, and swelled galls darken later. Active galls are mostly soft and flabby (rather than being hard); they get decayed in centers when aged. An important characteristic of the disease is to give no exudate on tumors, unlikely the other bacteria do in walnut (Figure 10). In young trees, the growth process can be affected remarkably according to the severity of galling, while in older trees wood-rotting is a prevalent occurrence. Care should be taken not to confuse the symptoms of this disease with galls caused by insects or nematodes.

Figure 10. Tumors on Older Walnut Trees (Photo: https://www.sjvtandv.com/)

Nurseries are the first risky sites that provide desired conditions in favor of agrobacteria activity (Figure 11). The pathogen may enter the soil (through organic residues for instance) and reside there for 1-2 years and can be detected on roots regardless of being host or not. Since the entrance of pathogen is only possible through wounds, subjecting to mechanical injury (during seedling transferring or any horticultural practices) is regarded as a potential risk for disease. Uncontrolled infected plant materials most possibly result in the dissemination of inoculum to the orchards (Teviotdale et al. 2002).

Figure 11. Characteristics Galls of *A. tumefaciens* on Young Plants (Photo: https://www.growingproduce.com)

Control of disease: *A. tumefaciens* is on the quarantine list in most countries, and strong precautions are taken by nurseries to produce clean plant materials. Moist control in orchards is essential for reducing the contamination risk and development of crown gall. It is necessary to use healthy seedlings for setting up an orchard. On the other hand, it is needed to abstain from activities resulting in physical wounds for planting. Although preventive biological control agents (not eradicating) are available to root dip application before transferring plant materials, their efficiency can be different on walnut trees. In the possible use of diseased plant materials, a combination of some treatments such as doing surgery, flaming, and applying bactericide are suggested in older trees only. It should be taken into consideration that the success of operations is extremely limited and expensive. Plant stunting should be removed completely from the orchards. Crop rotation (especially with grass) may help degrade remaining host materials and reduce the pathogen dispersion.

Phytophthora Root and Crown Rot

Several *Phytophthora* species cause root and crown rot of walnuts. General symptoms of the disease are necrosis of the collar and main roots; dark, flame-shaped necrosis, spreading up into the trunk (Belisario et al. 2006).

Phytophthora infection generally progresses in roots or crown, and the trees decline after the moderate spring season. Symptoms on the trees depend on infection rates on roots or crown (Figure 12). Generally, infection of fungi makes progress a couple of years. At the beginning of the disease, the growth of trees is reduced, followed by more severe symptoms in subsequent years gradually. Defoliation and dieback can occur in the final years in the condition of no precautions for disease.

Figure 12. General Symptoms of Phytophthora Disease on Walnut Trees

(Photo: https://www.sacvalleyorchards.com)

Control of Disease: The first principle of controlling the disease is to prevent the crown from getting wet and also to avoid waterlogging of soil around the crown with the duration of 18-24 hours, which favors for *Phytophthora* infections. On the contrary, water drainage in orchards reduces the risk of the causal agent. Water management in orchards, limitation to 18 hours for irrigation is highly recommended for preventing of spore production of fungi. The susceptibility of walnut cultivars or rootstocks against the fungi is varied. However, none of them are resistant to all *Phytophthora* species causing crown or root rot. Although the existence of some fungicides against the disease, the resistance of *Phytophthora* species in different cultivars are taken into consideration in walnut.

Botryosphaeria and Phomopsis Cankers

Cankers on walnut are described by two groups of fungi, which are *Botryosphaeria dothidea*,
Diplodia mutila, *D. seriata*, *Dothiorella iberica*, *Lasiodiplodia citricola*, *Neofusicoccum mediterraneum*, *N. nonquaesitum*, *N. parvum*, *N. vitifusiforme*, *Neoscytalidium dimidiatum*, belonging to the family Botryosphaeriaceae and *Diaporthe neotheicola*, and *D. rhusicola*, belonging to the family Diaporthaceae (Chen et al. 2013).
Botryosphaeria is accepted as an endophytic fungus in plants (Slippers and Wingfield, 2007) may cause disease in several woody plants (Chen et al. 2013). Generally, this fungus makes infection because of stress conditions (Blodgett and Stanosz, 1997). Disease

symptoms develop rapidly on the condition that stress factor is prolonged.

Shoot wilting and leaf flagging on branches are regarded as the initial symptoms (Figure 13). Brown or black color on cambium tissues is obvious under the bark. In the progress of the infection, pycnidia (containing fungal spores) or perithecia of both species can be detected under the bark. Shoots of young trees commonly get black after infection, while branches of older trees might die. *Botryosphaeria* fungi may colonize after infection of branch wilting fungus, *Neoscytalidium dimidiatum* (=*Scytalidium dimidiatum*, *Hendersonula toruloidea*), which has similar symptoms to cankers.

Figure 13. Initial Symptoms of Botryosphaeria Canker in Walnut Orchard (Photo: https://ccfruitandnuts.ucanr.edu)

Symptoms of disease are also remarkable on nuts. When the fruits become mature (during August or September), decay on the outer part of the nut is seen (Figure 14). The initial symptoms come in black color, then turn into brown to beige, and subsequently drop down prematurely. Contamination occurs from one fruit to another and thus, the disease spreads all through the tree.

Figure 14. Special Characteristic of Botryosphaeria is to Embroil Peduncle and Result in Nut Decay (Photo: https://ccfruitandnuts.ucanr.edu)

Botryophaeria and *Phomopsis* fungi usually make infection via wounds or prune areas by spreading spores on previously infected tissues in the tree, while direct infection occurs in favorable conditions.

Control of Disease: Cultural practices such as the pruning of infected shoots, twigs, etc. are essential to control the disease, and it also affects the efficiency of the chemical application. To prevent infections, protective fungicides should be applied for penetrating the inoculum through wounds or pruned areas. Registered fungicides in different countries can be applied in May or early summer to control the disease. Frequent precipitation in spring and temperatures over 10 ^{0}C are favorable conditions for disease. Because the success of chemical management is highly dependent on climatic circumstances, the weather conditions during spring such as heavy rainfall and moderate temperatures should be taken into account, therefore repeating of chemical application may be needed.

Fusarium Wilting

Fusarium solani and *F. incarnatum* are causal agents of the disease. Apart from thousand cankers disease on walnut, *F. solani* is determined as the primary pathogen in the trees. Fusarium-related wilting has been demonstrated on newly established walnut orchards. It is reported that symptoms of Fusaria lack sprouting on buds after transplantation, several injuries are seen in roots, color changes can be detected on the woody part of the plants, root necrosis is obvious in young trees (Seta et al. 2004; Singh et al. 2011; Mulero-Aparicio et al. 2019), and dieback allies with the discoloration of vascular bundles of shoots and twigs in mature trees (Chen and Swart, 2007).

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Part IX

Important Pests of Walnut and Their Control

Dr. Naim OZTURK[1] and Dr. Remzi UGUR[2]

Introduction

Anatolia is the homeland of walnut (*Juglans regia* L.) as well as many fruit species. Walnut; It is a very valuable fruit and wood species with wild forms in Anatolia, deciduous in winter, up to 30 m in height, cultivated in almost every part of our country. While the world walnut production is approximately 4.5 million tons (Anonymous, 2021), Turkey has 9,875,000 fruit-bearing walnut trees and ranks 3rd in the world after China and the USA in terms of walnut production with 225,000 tons (TUIK, 2020. As with other fruit varieties grown in Turkey, in parallel with the increase in production in recent years, many harmful insect and mite species in walnut orchards that cause plant health problems and cause significant product loss (Uygun et al., 2010; Canıhoş et al., 2014; Anonymous, 2017; Çakır, 2018). To obtain higher quality and abundant products, many methods are used

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in the fight against walnut pests. One of these methods is chemical control. However, unconscious, indiscriminate and intensive use of drugs has brought along many problems such as deterioration of natural balance, human and environmental health, durability and residue. To minimize these problems caused by pests seen in walnut orchards and to obtain products of higher quality and market value; Attention should be paid to "Integrated Struggle Programs" in which all warfare methods are used together with quarantine measures applied meticulously as well as producer training. As it is known, chemical control is the last method to be applied in integrated control programs. Therefore, this method should not be preferred unless it is a necessity. If chemical control is obligatory, environmentally friendly and specific drugs should be preferred and spraying should be done at the right time and dose.

Table 1. Common properties of walnut pests

Common name	Species name	Order, Family
Codling moth	*Cydia pomonella* L.	Lepidoptera: Tortricidae
Walnut aphids	*Chromaphis juglandicola* (Kaltenbach)	Hemiptera: Aphididae
	Panaphis juglandis (Goeze)	
Walnut eriophyid mites	*Aceria erinea* Nalepa	Acarina: Eriophyidae
	Aceria avanensis (Bagdasarian)	
Leopard moth borer	*Zeuzera pyrina* L.	Lepidoptera: Cossidae
Pear lace bug	*Stephanitis pyri* (Fabr.)	Hemiptera: Tingidae
Walnut leaf miner	*Caloptilia roscipennella* (Hübner)	Lepidoptera: Gracillariidae

This study; It is aimed to contribute to the solution of phytosanitary problems in walnut orchards by giving brief information about the definition of the pests. The form of damage, and the control of their symptoms, as well as the practical information obtained from the studies on "Walnut Pests" in the walnut orchards of Turkey.

Important Walnut Pests and Control

Codling moth (*Cydia pomonella* L.)

The adults of the Codling moth (*Cydia pomonella* L.) are small and gray, with dark, transverse wavy lines on their front wings (Figure 1).

Figure 1. The adults of Codling moth (*Cydia pomonella* L.)

The mature larva is 15-20 mm long and has a whitish-pink appearance (Figure 2a). Butterflies that emerge in the spring usually lay their eggs on fruits and rarely on leaves and twigs close to the fruits (Figure 2b). Walnut fruits are phenologically the size of hazelnuts when the first moths lay eggs and the first damage occurs (Figure 3).

*

Figure 2. Codling moth's larval (a) and egg on fruit (b).

The pest usually spends the winter between the cracked bark of the tree trunk and the fallen plant remains, inside the fruits, in the storage houses in the mature larval stage. The first butterflies start in April-May and continue until July. The second-generation adults are seen in July-August. For the adults to mate and lay eggs, the twilight temperature must be above 15 °C two evenings in a row (Anonymous, 2017).

Figure 3. First offspring damage and larval entry sites of codling moth

Larvae that emerge from the eggs usually enter from the parts where the fruits come into contact with each other or the leaves, the stem base, the flower pot, and the fruit sides (Figure 4). Young larvae may die at a high rate before they enter the fruit (because they are very sensitive to adverse conditions such as wind, rain, cold, etc., or due to natural enemies). Since it is essential to kill the larvae before they enter the fruit, it is significant in the fight against the pest during this period. The larva, which matures by feeding in the fruit for 30-40 days, leaves the fruit from the hole it entered and becomes a pupa in a suitable place.

The Codling moth (*Cydia pomonella* L.) directly harms the walnut fruit. The larva feeds on the inner and outer shell of the walnut, causing the fruit to fall, reducing the quality and market value of the product. The first generation is fed in the fruit, and the second generation is usually fed on the peel. The first progeny larvae, which enter through the green shell of the walnut, open a gallery and pierce the inner shell, which is not yet hardened, and feed inside the fruit, while the second progeny feed mostly on the green shell and open a gallery under the shell (Figure 5). However, since they cannot pierce the hardened shell during this period, they cannot enter and there is no damage to the fruit. In varieties with an opening in the fruit shell, the second generation larvae can enter through the hole in the fruit stem pit or the opening at the junction of the peel, feed in the fruit and cause damage.

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Figure 4. Fruit entry points and damage of Codling moth (*Cydia pomonella* L.)

The Codling moth (*Cydia pomonella* L.) is a polyphagous pest. Walnut, apple, pear and quince are among the important hosts. In addition, it has been determined that it rarely harms plum, apricot, peach and wild berry.

Figure 5. The damage of Codling moth (*Cydia pomonella* L.) in walnut fruit.

Control

Cultural measures: It should be preferred to establish a closed walnut orchard and care should be taken not to plant fruit trees such as apple, pear and quince, which are the other hosts of the Codling moth (*Cydia pomonella* L.), in and around the garden. Contaminated fruits spilled on the ground throughout the year should be collected and destroyed weekly (Canıhoş et al., 2014; Anonymous, 2017).

Biological control: The apple wolf has many natural enemies in nature. First of all, care should be taken to increase the efficiency of these species by protecting them.

For this, random drug applications should be avoided, and when necessary, specific drugs with low impact on the environment and natural enemies should be used. Perennial shelter plants (wild rose, blackberry, etc.) and flowering plants such as mint, wild carrot and fennel, which can be a food source (prey, pollen, nectar, etc.) for natural enemies, should be found or grown in the garden edges.

Biotechnical control: The technique of inhibition of mating is successfully applied in the control of the codling moth (*Cydia pomonella* L.) (Figure 6).

Figure 6. Codling moth (*Cydia pomonella* L.) trap and pheromone dispenser.

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Chemical control: The aim of the fight against the codling moth (*Cydia pomonella* L.) is to kill the larvae before they enter the fruit. In walnuts, the first generation causes economic damage and the second generation does not cause any harm in general. However, in some cultivars, spraying may be required for the second generation, as the bark is not completely closed and the opening remains and the progeny are mixed. For this purpose, in general, when the fruits are phenologically hazelnut-sized, and 15 days after that, 2 sprayings can be done for the first generation, and if necessary, 1 spraying can be done for the second-generation 15 days after the second spraying.

Walnut aphids [*Chromaphis juglandicola* (Kaltenbach) and *Panaphis juglandis* (Goeze)]

Walnut aphids are generally lemon yellow or yellowish greenish. *C. juglandicola* scattering colonies under walnut leaves and feeding (Figure 7a). P. juglandis species feeds by localizing along the midrib on the upper surface of the leaves (Figure 7b). Both species spend the winter in the egg stage. All of the individuals that hatch in the spring consisting of female individuals, reproduce without fertilization (fertile) throughout the summer and give a large number of offspring per year (Anonymous, 2017).

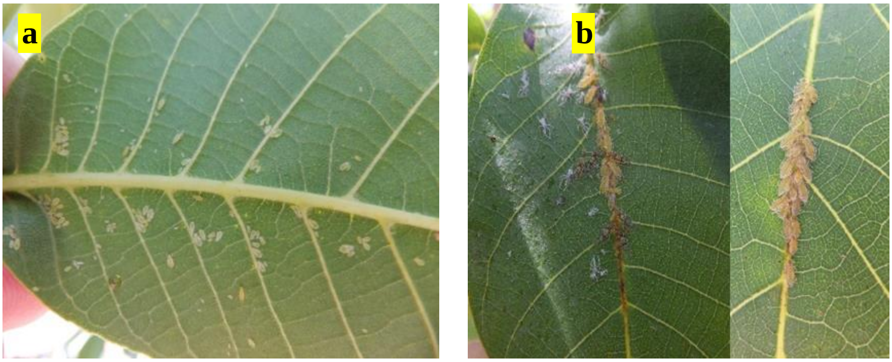

Figure 7. The under and upper leaf appearance of *Chromaphis juglandicola* (a) and *Panaphis juglandis* (b)

Walnut aphids; It feeds on the leaves and fruits of the walnut by absorbing the plant sap. As a result of absorption, photosynthesis is adversely affected by the breakdown of chlorophyll, yellowing of leaves, and shedding in dense populations. In addition, fumagin is formed on leaves and fruits due to the honey-like substance they secrete (Figure 8). This situation causes weakening of the tree, deformation of the leaves, decrease in product quality and yield.

C. juglandicola and *P. juglandis* species often coexist in the plant organs they feed on. If the presence and damage of aphids will continue in the near-harvest period at the end of summer, shriveling will occur in the inner part of the fruit and the quality will decrease due to this situation (Canıhoş et al., 2014). Of the walnut aphids, P. juglandis is a monophagous species. On the other hand, *C. juglandicola* species, *Ricinus communis*, *Crataegus* sp., and *Prunus amygdalus* (Canıhoş et al., 2014; Anonymous, 2017).

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Figure 8. Fumagin damage of walnut aphids on leaves and fruits

Control

Cultural measures: Aphids; It is more of a problem in gardens that are planted frequently, are not regularly pruned, have high humidity, and use excessive and broad-spectrum insecticides. For this reason, frequent planting, excessive irrigation, and nitrogen fertilizer application should be avoided in new plant gardens. Care should be taken to clean weeds in and around the garden, and trees should be pruned regularly every year to ensure airflow in the garden.

Biological control: Walnut aphids have many natural enemies in nature. First of all, care should be taken to increase the efficiency of these species by protecting them. For this, random drug applications should be avoided, and when necessary, specific drugs with low impact on the environment and natural enemies should be used. Perennial shelter plants (wild rose, blackberry, etc.) and flowering plants such as mint, wild carrot, and fennel, which can be a food source (prey, pollen, nectar, etc.) for natural enemies, should be found or grown on the garden edges. In addition, ant exits to trees should be prevented to feed on the honey-like substance secreted by aphids.

Chemical control: In general, a single application in the spring is sufficient against walnut aphids. For this purpose, 20 trees are determined to represent the garden in the early period in the gardens known to be infected from the previous year, and 5 leaves are taken from each tree, from different directions and heights. When the density of 15 individuals/leaf per 100 leaves taken is determined, spraying is done. If necessary, 2-3 more sprayings can be done according to the effective time of the drug used in the summer months (Canıhoş et al., 2014; Anonymous, 2017).

Walnut eriophyid mites [*Aceria erinea* Nalepa and *Aceria avanensis* (Bagdasarian)]

Adults of Walnut Eriophyid mite are yellowish-cream colored, transparent and carrot-shaped. These species are very small, about 0.1-0.3 mm in size, and move slowly (Figure 9). However, they are known for the damage they cause to walnut leaves and fruits.

Winter, They live in buds, under the bark, in slits and cracks in the stem. Adult individuals start to feed on young leaves and fruits by leaving the winter when the buds start to appear in the spring. Then they lay eggs in the gall and ridges they have formed on the leaves and fruits.

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Adult individuals leave the ridges and galls and migrate to young leaves and fruits. They do the main damage towards the end of the summer season when they are at their peak. They are generally mechanically transported by wind, birds, and insects. In addition, sapling transportation plays an important role in the transportation of these species to clean areas. They give many offspring per year (Canıhoş et al., 2014; Anonymous, 2017).

Figure 9. The appearance of nymphs and adults belonging to *Eriophyid* mite species

Walnut Eriophyid mites feed by sucking the sap on the leaves and fruits of the walnut. The toxic substance they secrete during sucking causes deformation (gal, blistering and spalling) in the plant tissue. A. erinea species feed only on leaves and causes large galls on the upper surface of the leaf and perineum (hairs) on the lower surface (Figure 10). A. avanensis, on the other hand, feeds on the leaves and fruit of the walnut, causing wart-shaped small galls on the lower and upper surfaces of the leaves and fruit, and deformation in the form of incrustation on the bark (Figure 11). The gall and ridges, which are first light greenish, gradually turn red, brown, and black. In heavy contamination, the leaves fall prematurely, the shape of the fruit deteriorates and its quality decreases.

Walnut Eriophyid mites are monophagous species and no hosts other than walnuts have been encountered so far (Canıhoş et al., 2014; Anonymous, 2017).

Figure 10. Damage of *Aceria erinea* (blister mite) species on walnut leaf upper and lower surfaces

Figure 11. Damage of *Aceria avanensis* (gall mite) species on walnut fruit and leaf

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Control

Cultural measures: Saplings and cuttings to be used while establishing a garden should not be contaminated with pests. Annual maintenance of the garden should be done regularly, trees should be kept healthy and excessive nitrogen fertilizer use should be avoided. Infected shoots should be pruned at the end of spring, and leaves that fall to the ground in autumn together with pruning residues should be collected and destroyed. In addition, weed control in and around the garden should be done regularly.

Biological control: Eriophyid mites have many effective predators in nature. First of all, care should be taken to increase the efficiency of these species by protecting them. For this, random drug applications should be avoided, and when necessary, specific drugs with low impact on the environment and natural enemies should be used. Perennial shelter plants (wild rose, blackberry, etc.) and flowering plants such as mint, wild carrot and fennel, which can be a food source (prey, pollen, nectar, etc.) for natural enemies, should be found or grown in the garden edges.

Chemical control: In the gardens known to be contaminated with pests in previous years, leaves and fruits are checked in spring. Galls, which are signs of nutrition, are searched, and when signs of damage are seen on leaves and fruits, pesticides are applied. However, in the gardens where the contamination is intense every year, as the appropriate spraying time; It is the April-May period in which the adults migrate to the leaves and fruits of the plants where they spend the winter in the spring and the October-November period when they migrate to their hosts to spend the winter again in the autumn.

In nursery gardens and young gardens (under 5 years old), at least one spraying should be done when damage is observed. If the damage continues on the newly emerging leaves, another spraying can be recommended considering the effect time and damage status of the drug (Canıhoş et al., 2014; Anonymous, 2017).

Leopard moth borer [*Zeuzera pyrina* L.)

Adults are large and have a wingspan of approximately 41-43 mm. Its wings are white with dark spots on the wings (Figure 12a). The larva is large and dark yellowish with dark dotted spots (Figure 12b).

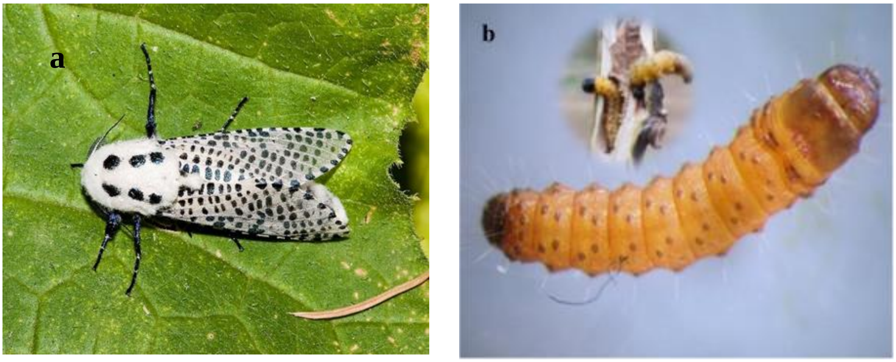

Figure 12. Adult (a) and larva (b) of Leopard moth borer [*Zeuzera pyrina* L.).

The overwintering larvae of the woodworm start to feed in March-April and juice flows from the gallery openings (Figure 13a). The first butterfly flights are seen in May-June. Butterflies are active at night and lay eggs (Figure 13b). Eggs; It is usually left in groups near the

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adult exit holes, in the cracks on the stem, and between the bark (Canıhoş et al., 2014; Anonymous, 2017).

Figure 13. Feeding sign of Leopard moth borer [*Zeuzera pyrina* L.) larva - sap discharge (a) and eggs (b).

The larvae open a gallery on the annual shoots, branches and trunks of the walnut. Feeding scum (Figure 14a) and plant sap flow are present at the mouth of newly entered galleries (Figure 14b). Due to the opened galleries, the transmission bundles in the tree branches and trunk are damaged and the transmission of water and nutrients to the upper parts of the plant is prevented. Therefore, there is a sign of growth retardation and a general stagnation in the plant. Contaminated trees and branches can dry completely in about 2-3 years. Opened galleries cause breakage in twigs or saplings due to windy weather or fruit load (Canıhoş et al., 2014; Anonymous, 2017).

Figure 14. Gallery mouth feeding waste (a) and plant sap discharge (b) of Leopard moth borer larva.

This type of damage causes yield loss and distortion of the crown shape. The sap flow from the larval entrance holes is typical of this pest (Figure 13a, 14b). Z. pyrina is mostly a problem in old, neglected, barren and high-ground water gardens.

The pest usually gives offspring once a year and sometimes once every two years.

The leopard borer moth is a polyphagous pest. It is harmful to forest trees and shrubs, usually fruit trees. Walnut, pomegranate, olive, apple, pear, quince, cherry, plum are among the important hosts.

Control

Cultural measures: Steel and saplings to be used while establishing a garden should not be contaminated with pests. Garden maintenance should be done well, trees should be kept healthy and attention should be paid to weed cleaning, especially under the tree crown. Trees should be pruned regularly every year, and the infected branches

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should be cut and destroyed both during pruning and during the controls during the year. In orchards with low density, the larvae should be killed by inserting steel wire into the galleries or injecting a suitable insecticide.

Chemical control: Since the larvae of the pest feed in the gallery in the wood tissue of the tree, it is very difficult to control. In May-June, the first spraying should be done 7-10 days after the first butterfly is seen, probably in June. If the butterfly flights continue, one more spraying can be done 20-30 days after the first spraying, possibly in August and October (Canıhoş et al., 2014; Anonymous, 2017).

Pear lace bug (*Stephanitis pyri* (Fabr.))

In adults, the body is dark-colored, flat and wide, and it is an ornamental insect. When viewed from above, its wings are transparent and have a bee-comb pattern (Figure 15a).

Figure 15. Adults and nymphs of the pear lace bug (a) and feeding waste (b).

Winter; They spend their adult life under tree bark, in soil crevices and roofs between stones, and under dried leaves. As of April, they leave their winter quarters and move to fruit trees. It lays its eggs one by one in the tissue under the leaf. The eggs are covered with a sticky liquid such as pitch for protection (Figure 15b).

Adults are withdrawn to winter from October and the pest gives 2-3 offspring per year.

The nymphs and adults of the pear tiger suck the sap of the leaves, causing the chlorophyll to break down and yellowish-white spots to form on the leaf (Figure 16). As a result of feeding on the underside of the leaf, dark-colored scum accumulated in small spots and the honey-like substance they secrete prevents the leaf from respiration and causes drying and shedding starting from the leaf tips. In high populations, growth retardation occurs in trees, shoots cannot fully mature, fruits remain small, so the quality and yield of the product decrease.

Figure 16. Nutritional damage and symptoms of the Pear Lage Bug on the leaves

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The pear lage bug is a polyphagous pest. Walnut, pear, apple, quince, cherry, cherry, peach, apricot, plum, chestnut, medlar, hazelnut, blackcurrant, poplar, willow, elm, plane tree and ornamental plants are among its important hosts (Canıhoş et al., 2014; Anonymous). , 2017).

Control

Cultural measures: frequent planting should not be done in new plant gardens. Excessive irrigation and nitrogen fertilizer application should be avoided. Care should be taken to clean weeds in and around the garden. Trees should be pruned regularly every year and airflow should be provided in the garden.

Biological control: The pear tiger has many natural enemies in nature. First of all, care should be taken to increase the efficiency of these species by protecting them. For this, random drug applications should be avoided, and when necessary, specific drugs with low impact on the environment and natural enemies should be used. Perennial shelter plants (wild rose, blackberry, etc.) and flowering plants such as mint, wild carrot and fennel, which can be a food source (prey, pollen, nectar, etc.) for natural enemies, should be found or grown in the garden edges.

Chemical control: As of April, 3 leaves are checked from one shoot in 4 different directions of 10 trees determined to represent the garden. If the average number of adults is 1 individual/leaf or more in the counts, spraying is done. If the number of nymphs+adults is 4 individuals/leaf in the controls made in June, second spraying may be recommended (Canıhoş et al., 2014; Anonymous, 2017).

Walnut leaf miner (*Caloptilia roscipennella* (Hübner))

C. roscipennella is a micro butterfly species. Adults range in color from grayish-white to light brownish gray or dark yellow to reddish-brown (Figure 17a). There are silvery blackish spots on the wings and fringe-shaped hairs that vary from yellow to black on the edges (Canıhoş et al., 2014).

Larva; It's 9 mm long, the yellowish-green body is transparent and its intestines are visible (Figure 17b).

Figure 17. Adult (a) and larva (b) of the walnut leaf miner

It spends the winter in the sheltered parts of the tree in the adult period. *C. roscipennella* is a monophagous species and feeds on the leaves and shoots of walnuts. It lays its eggs on the leaves of trees. The larva opens a gallery in the leaf and causes curling in the form of a roll. In dense populations, leaves and shoots dry out (Figure 18). This damage is more important in nurseries and young gardens, it

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prevents seedling growth and leads to deterioration of the crown structure. In older trees, this damage can be tolerated with copious shoot yields. The feeding damage of the larva on the leaves is first seen in April. The density and damage of larvae in the shoots occur mostly in the months of June-July (Canıhoş et al., 2014).

Figure 18. The damage of walnut leaf miner on walnut leaves.

Control

Cultural measures: Excessive irrigation and nitrogen fertilizer application should be avoided. Trees should be pruned regularly every year and airflow should be provided in the garden. The gluttonous shoots and pest-infested shoots on the tree should be cut and destroyed during the year.

Biological control: C. roscipennella has many natural enemies in nature. First of all, care should be taken to increase the efficiency of these species by protecting them. For this, random drug applications should be avoided, and when necessary, specific drugs with low impact on the environment and natural enemies should be used. Perennial shelter plants (wild rose, blackberry, etc.) and flowering plants such as mint, wild carrot and fennel, which can be a food source (prey, pollen, nectar, etc.) for natural enemies, should be found or grown in the garden edges.

Chemical control: In the control of C. roscipennella, there is no need for additional spraying because the damage is tolerated on large trees and the pesticides applied against other walnut pests also control this pest. However, since the damage is more important in the nurseries, 1 spraying is done when the leaves and shoots show signs of damage during the controls to be made in April-May. If necessary, 1-2 more sprayings can be recommended after 15-20 days, taking into account the effect time of the drug.

Result

In Turkey, many closed walnut orchards have been established due to the increasing domestic and foreign market demand, especially after the 2000s. This situation has brought along many plant protection problems as well as aquaculture problems. Along with these problems, human and environmental health should be prioritized against possible plant health problems in the future, and more importance should be given to "Integrated struggle and "Organic agriculture", which are agricultural techniques that protect and support the natural balance. While planning these studies, the agro-ecosystem should always be considered as a whole and sufficient information should be obtained about the important pests seen in walnut orchards and their control. In addition, to leave a healthier environment for future generations with sustainable agricultural activities, efforts should be made to preserve

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the natural balance in the ecosystem by using environmentally friendly-specific drugs with the right techniques at the right time, instead of intensive pesticide applications in herbal products.

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Part X

Walnut (*Juglans regia* L.) Breeding and Walnut Breeding Criteria

Prof. Dr. Halil İbrahim OĞUZ[1], İlbilge OĞUZ[2]

1. Introduction

Considering the distribution and habitat of walnuts throughout the world; it is a type of fruit that spreads over a wide area from the Carpathian Mountains, from the South to Eastern Europe, and from Turkey, Iraq, east of Iran to the Himalayan Mountains. Although there are many species of walnuts, the most commercially produced and consumed species in the world is *Juglans regia L.*, which is generally diploid with a chromosome number of 2n=2x=32. In addition, there are wild forests of *Juglans regia L.* especially in the western border mountains of Kyrgyzstan, Tajikistan, Uzbekistan, and China. (Akça, 2001; Şen 2005; Guney et al., 2021).

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As is known, after understanding the importance of walnuts in the nutritional benefits of walnuts for human health, interest in walnut production has increased rapidly in the world. According to the data of FAO for 2019, world walnut production is 4.498.442 tons, of which 2.5021.504 tons are produced by China alone. In terms of walnut production, the USA ranks second with 592.390 tons, Iran ranks third with 321.074 tons, and Turkey ranks fourth with 225.000 tons. In recent years, both the production area has been expanding and all walnut producing countries have been making efforts to increase the yield per hectare. Besides, the export of shelled walnuts in the world increased from 272.705 tons to 335.361 tons in 2016. Regarding the exports of walnuts in shell, the USA alone ranks first 129.827 tons, Mexico ranks second with 50.029 tons, Chile ranks third with 32.014 tons followed by Ukraine with 28.594 tons, China with 23.148 tons, Germany with 14.489 tons, Moldova with 11.915 tons and Turkey with 6.248 tons. Although Turkey is the fourth country in terms of walnut production in the world, its exports fall far behind. The reason for this is that the majority of the walnuts produced find buyers in the domestic market at high prices. Another country that draws attention to exporting walnuts is Germany. Germany creates awareness by buying shelled walnuts cheaper than the world walnut market from countries with low marketing opportunities, packaging them in its own country and then exporting to the European and world walnut market. The value of exported walnuts worldwide amounted to 2.246.631 on a dollar basis. The USA alone meets 37.02% of this value. This is followed by Mexico City with a share of 21.9%, Chile 9.2%, Germany with 6.2%, China with 5.2%, Ukraine with 3.15%, Moldova with 2.3%. The rest is exported by other countries. Once more, according to the data of FAO for 2019, Walnut global import increased from 1.389.795 tons in 2016 to 1.722.778 tons in 2020. Germany is the leading country with 317.843 tons in terms of importing shelled walnuts. Regarding the walnut in shell imports, Japan ranks second with 136.993 tons, Spain ranks third with 121.628 tons followed by Canada with 84.916 tons, South Korea with 82.659 tons, England with 78.509 tons, France with 71.935 tons, Italy with 59.517 tons, the United Arab Emirates with 43.869 tons and Turkey with 16.615 tons. Here, there has been a decrease in Turkey's walnut imports in recent years, which can be expressed as a result of the increase in newly established walnut plantations in Turkey (FAO, 2019; TUIK, 2020).

In recent years, walnut production areas in Turkey show a rapid increase with the support and developments in grafting and grafted sapling production. In addition, some of the important reasons for the increase in production can be explained by the price stability, the fact that walnut production is easier than other fruit types, and has fewer marketing challenges. However, the production target has not yet been achieved due to problems such as choosing the right variety, choosing a planting site, preparing the land for planting, planting methods, and garden management. As it is known, there are many walnut genotypes that have been propagated by seed, have a very high genetic diversity, and have a high preferability value in terms of breeding. Considerable selection studies have been carried out in each walnut production area throughout Turkey, and many genotypes which are suitable for breeding have been selected (Akça, 2001; Şen, 2005, Oğuz and Aşkın, 2007; Keles et al., 2014; Kafkas et al., 2020; Arcan et al., 2021). During the selection studies carried out after the 1970s, criteria including late leafing, fruit weight, kernel weight, shell thickness, resistance to diseases and pests, non-periodicity, lateral bearing habit, kernel color, and kernels which are easily removed from shell were emphasized in general. Researchers working on walnut breeding in Turkey have chosen high-yielding genotypes by making point selections in many regions of Anatolia for many years. Unfortunately, some of these selected genotypes could not be propagated by grafting. However, although these selected genotypes are reproduced and protected by public projects in orchards established in different regions, the programmed and interdisciplinary walnut breeding studies are not sufficient in Turkey. Although there are many researchers working on walnut breeding, due to the lack of cooperation, teamwork, and national walnut breeding program, walnut breeding studies have unfortunately not reached the desired level.

This study was conducted in order to specify in detail the historical background of walnut breeding studies in the world, breeding studies by country, outstanding criteria in breeding studies, methods used in breeding studies, cultivar and rootstock breeding, developments in breeding methods in recent years and future breeding strategies.

2. AN OVERVIEW OF WALNUT BREEDING

So as to define the selective breeding method briefly, it is a breeding method performed by selecting plants suitable for breeding purposes and criteria from natural populations which are rich in diversity and giving them the chance to get more yield. As it is known, the basis of plant breeding is based on selective breeding. However, this method is based on finding new promising genotypes by selecting genotypes suitable for their purpose from existing variations, rather than creating genetic variation. On the other hand, in other breeding methods, artificial variability is created through hybridization, in other words, variations are created so that the selection is made from these variations. In selection breeding, the hereditary structures of plants are preserved. Thanks to this feature, selection breeding can influence the allele frequencies in a population. In other words, the population's genetic variance can be shifted toward the desired direction. Success in selection breeding depends on the genetic variability, the source of variability, and exploitation of variability in the studied populations. In brief, the selection technique to be used is important. The source of variation in a population is caused by multiple factors. For example, one cause is due to environmental conditions (modification), and the other is due to hereditary structure (idiovariability). The higher amount of genetic variance in the total, the higher the effect and accuracy of selection. While the selection criteria to be used in selective breeding should be considered separately for each ecology, some characteristics such as lateral bearing trait and yielding, periodicity, fruit characteristics, resistance to diseases and pests, quality, early fruiting, growth strength, and morphological structure of the tree should be included among the breeding objectives. In line with these objectives, the main characteristics such as fruit quality, yielding, tolerance to disease, and pest were emphasized as selection criteria in the study on the breeding of walnut in the Marmara region by the selection, which was the first walnut breeding study in Turkey. At the same time, this study has made a very important contribution to future studies both as it was the first breeding study conducted and as it used the selective breeding method (Germain, 2004; Akça, 2005; Öztürk, 2021).

In general, the aim of walnut breeding is to breed new varieties with high yield, resistance to diseases and pests, biotic and abiotic stresses. In 1948, the first breeding study was carried out in France and the USA using the selection method. High-quality genotypes are selected from countries such as Iran, China, Afghanistan which are the homelands of the walnut, and reproduced in countries such as France, China, Turkey, Iran, Spain, Italy, and the USA, and thus new variations were obtained by natural hybridization methods. Later, breeding methods were developed, as a result, phenotyping and new molecular breeding methods were started to be used. In recent years, developments in genomics and biotechnology have led to significant advances in the walnut cultivar and rootstock breeding. In addition, in the future, skilled biotechnologists can identify and develop desirable traits for breeding in a suitable gene bank, and long-term germplasm conservation of biodiversity can be ensured.

As a result, the use of molecular breeding and techniques of biotechnology can play a decisive role in countries such as the USA, China, France, Iran and Turkey, which carry out walnut breeding studies. These techniques are high-throughput genotyping platforms, genomic-based approaches, genome-wide association studies, marker-assisted selection, genomic selection, and next-generation sequencing techniques such as genomic editing with the CRISPR-Cas9 system, bioinformatics tools. Thanks to these techniques, fast and clear results will be obtained in walnut production, variety and rootstock breeding. In our recent past and even today, many producers use rootstocks propagated by seed in walnut production. Walnut producers in many parts of the world use new varieties developed by leading countries in walnut production. For example, the Chandler walnut variety is one of the most produced varieties in the world, for its lateral bearing habit, productivity and white-colored kernel (Tulecke and McGranahan 1994). Franquette is a late leafing variety. This variety is widely produced in the world as well. However, although it was a common variety especially in France, it is now being replaced by Fernor and Chandler varieties (Germain 1999; Vahdati, et al., 2019). It is always difficult to achieve specific goals in plant breeding. Yet, the main goal for all kinds of products is to provide high yield and quality. Regarding walnut breeding, first thing is to focus on criteria such as nut size, kernel color, kernel weight, a kernel that is easily removed from the shell, and shell thickness. In addition to these, the morphological development of the trees, lateral bearing habit as the major criterion of yield were accepted as the dominant criteria in the breeding of commercial varieties such as Chandler in the middle of the twentieth century. Besides criteria such as resistance to diseases (blight, anthracnose) and late leafing were emphasized as well. Some characteristics of walnuts also vary depending on the ecological and climatic conditions in which they are produced. For example, in countries such as Turkey, France and Iran, which are adversely affected by climatic conditions, late leafing is an important breeding criterion due to late spring frosts (Avanzato et al. 2014; Bernard et al. 2018). In addition, late leafing has gained importance in resistance to walnut blight (*Xanthomonas arboricola pv. juglandis*), which causes widespread damage, especially in spring during high rainfall. Earliness in walnut production has been accepted as an important criterion to avoid autumn rains and therefore to determine the harvest time correctly (Akca and Ozongun 2004; Bernard et al. 2018; Ebrahimi et al. 2015; Germain 1989; Leslie and McGranahan 2014). Moreover, it is believed that criteria such as biotic and abiotic stresses, tolerance to drought, resistance to diseases and pests, tolerance to climate changes and global warming will be regarded as goals in walnut breeding in the future (Vahdati, et al., 2019).

3. WALNUT BREEDING PROGRAMS WORLDWIDE

3.1. Variety Breeding

The history of walnut variety breeding programs throughout the world is based on the recent past. Variety breeding has started with the genetic development of walnuts and the with the grafting and propagation of walnut genotypes, which are considered superior within the natural population by farmers. Squirrels and crows have had a huge impact on increasing variation in natural populations of walnuts. Since these animals forgot most of the seeds they buried in the ground to store food in the winter, they caused many new walnut variations to be formed by the germination of these seeds in the spring (Akça, 2001; Şen, 2005; Vahdati and Rezaee, 2014). In order to summarize the developments in walnut variety breeding, it will be sufficient to examine it in 4 stages.

The walnut breeding studies should be listed generally in the time order; from 1700-1948, 1948-1979, 1979-2009 and 2009 to the present. The first studies in walnut variety breeding started in France with the selection and grafting of superior genotypes and their distribution to the producers as seedlings. By this means, varieties such as Franquette, Mayette, Crone, Grandjean and Parisienne were obtained. In the United States, the first breeding studies were carried out with this method as well. The source of walnut breeding material in the USA consists mostly of *Juglans regia L.* seeds originating from Iran and Afghanistan.

The first breeding and selection studies in the USA were made by Felix Gillet in Northern California between 1835-1908. Then these studies were conducted by Josef Sexton (1842–1917), a walnut grower in Southern California between 1842–1917. Eureka, Waterloo, Poe and Hartley American walnut varieties were selected by these researchers. Moreover, California farmer George Payne selected the Payne variety and produced it as an important walnut variety in

California for many years. In addition, the Payne variety has been an important genetic resource in American walnut breeding (Ramos 1997; Tulecke and McGranahan 1994). At the same time, it is claimed that the source of the Eureka walnut variety in the USA originates from Iran and Afghanistan. Walnut breeding studies in the USA and France accelerated the hybrid studies, which is the second stage in the world walnut breeding. In particular, crossing studies have been initiated between genotypes propagated with seeds imported from natural walnut populations originating from the Silk Road. In these hybrid studies, lateral bearing trait has been regarded as the dominant criterion. Eugene F. Serr and Harold I. Forde carried out this breeding phase from 1948 to 1979 and as a result of the breeding studies, walnut varieties such as Midland, Vina, Pioneer, Pedro, Gustine, Lompoc, Amigo, Chico, Tehama, Serr, Chandler, Howard and Sunland were developed. Among these cultivars, Chandler, Serr and Howard cultivars became the most popular walnut cultivars in the world. Especially the Chandler variety constitutes 72% of the walnut orchards in California, while the Sert variety constitutes 12%. In these years, the targeted breeding criteria were late leafing, lateral bearing habit, earliness, strong tree structure, kernel quality and tolerance to diseases. Studies have shown that the parents and ancestors of Ser and Forde cultivars are Payne cultivars (Ramos 1997; Tulecke and McGranahan 1994; Vahdati and Rezaee 2014; Bernard et al. 2018).

In France, Eric Germain obtained 28 crosses (1900 intraspecific hybrids) between French and Californian cultivars at INRA in the second stage of the walnut breeding program. The hybrids obtained here were evaluated and as a result, Lara cultivar and Franquette cultivar were obtained. Later, Fernor variety with late leafing was developed in 1987. Here, again, late leafing and lateral bearing criteria were taken into account for breeding purposes (Bernard et al., 2018; Germain 1999; Ramos and Doyle 1984). The third and fourth walnut breeding stages in the world were carried out in Iran between 2009 and 2010, as well as in the USA and France between 1979 and 2009, and 2009 until today (Hassani et al. 2013). Regarding the fourth stage, criteria such as late leafing, lateral bearing trait and yielding were emphasized. Germplasm evaluation and hybridization studies are still ongoing. Currently, there are four walnut varieties as Persia, Hazar, Chaldoran and Alvand (Vahdati and Rezaee, 2014).

In addition to these, in Iran, germplasm evaluation, hybridization and molecular breeding studies continue under the management of Kourosh Vahdati at Tehran University. In these studies, varieties such as Kerman, Ilam, Fars, Qazvin, Alborz, Yazd, Kohgilooye and Boyer-Ahmad, Mazandaran have been developed. Criteria such as lateral bearing habit, early harvest, dwarfing, drought and stress tolerance are emphasized in these breeding studies, (Karimi et al. 2014; Vahdati and Mohseniazar 2016; Vahdati and Rezaee 2014; Vahdati et al. 2015).

Variety breeding studies in walnut are based on germplasm evaluation and hybrid breeding studies. Among these studies, germplasm evaluation studies are mostly carried out in Xinjiang province, where germplasm resources are abundant. About 80% of Chinese walnut cultivars were selected from Xinjiang germplasm and originated in Xinjiang province. The first walnut cultivar breeding studies in China were initiated in the early 1960s at the Liaoning Economic Forest Institute and the Chinese Academy of Forestry. However, existing walnut cultivars were later developed after uniform requirements for breeding criteria were established. Significant progress has been made in walnut breeding in China since 1980. Between 1979 and 2006, 26 new walnut varieties were bred. Baokexiang, Beijing 861, Jinglong 1, Jinglong 2, Lipin 1, Lipin 2, Lubo, Xifu 1, Xilin 1 and Xinzaofeng were among those varieties. All of these varieties have been developed through selection. In addition to these, Liaoning 1-8, Xiangling, Fenghui and Zhonglin varieties were also created as a result of controlled crosses. They reported that the most important walnut varieties developed as a result of controlled crosses were Xinjiang walnuts. Moreover, they reported that around 20 new walnut cultivars were added as a result of controlled crossing studies in the fourth breeding stage (Chen et al. 2014; Zhang et al. 2013a; Zhang et al. 2013b).

Most of the walnut varieties native to Turkey bear fruits on terminal buds. For this reason, in Turkey, breeding criteria are based on late leafing and fruiting on lateral branches. The walnut breeding program in Turkey is based on germplasm assessment and selection of superior genotypes. Although Turkey is the homeland of walnuts, the majority of walnut orchards consisted of genotypes propagated by seeds until recent years. Nevertheless, in the last 20 years, newly established commercial gardens have been established with standard varieties.

The first walnut breeding program in Turkey was initiated by Hayati Olez in 1971 in the Marmara Region. Later, selection breeding studies were conducted by many researchers in almost every region of Anatolia (Şen, in 1980 in North East Anatolia and Eastern Black Sea Region; Akça, in 1993 Gürün; Beyhan, in 1993 Darende; Akça and C. Ayhan in 1996; Yarlgaç, in 1997 Gevas: Oğuz, 1998 in Ermenek; Aşkın and Gün, in 1995 Çameli and Bozkurt; Yaviç, in 2000 Bahçesaray). At the same time, important genotypes have been identified in terms of breeding. In Turkish breeding studies, varieties such as Akça 1, Bilecik, Bursa 95, Kaplan-86, Şebin, Şen 2, Tokat 1, Yalova 1, Yalova 3, Yalova 4, Yavuz 1 were created through selection. (Akça, 1993; Akça and Ayhan, 1996; Akça and Özongun, 2004; Akça 2005a; ; Akça 2005b; Akça and Polat 2007; Askın and Gün 1995; Beyhan, 1993; Oguz, 1998; Ölez, 1971; Şen, 1980; Yarlgaç, 1997; Yavic, 2000). In addition to germplasm evaluation and selection studies, some controlled crosses between domestic and foreign varieties have been carried out in the walnut breeding program in Turkey since 2008. With these controlled crosses, approximately 1340 hybrid genotypes which are under evaluation were produced (Akça et al. 2016). Moreover, new Turkish walnut cultivars as Maraş 18, Sutyemez 1 and Kaman 1 were developed by means of germplasm evaluation in 2009 and 2010. Besides new varieties such as Diriliş, July 15, Maraş 12 and Bayrak are Turkish walnut varieties that have been introduced recently (Ozcan et al. 2017). The aim of the Turkish walnut breeding program is to develop new genotypes that are tolerant to salt, lime, drought and disease stresses and have characteristics of late leafing, lateral fruitfulness, early harvest (Ertürk and Akça 2014).

In addition to the countries mentioned above, walnut breeding studies are also carried out in countries such as Spain, Germany (Bollersen 2017), Georgia, Italy, Hungary, Greece, Romania, Ukraine, Serbia and Azerbaijan. In some of these countries, the walnut breeding program has started directly from the second stage. In these countries, genetic diversity and hybridization-based breeding studies are carried out within the framework of crossbreeding between French and California walnut varieties regarding the dominant criteria of breeding studies and as a result, local varieties were introduced (Avanzato et al. 2014; Vahdati et al. 2015).

3.2. Rootstock Breeding

As in all fruit growing, rootstock use is very important in determining productivity. Rootstocks affect the morphological and physiological development of the grafted culture plant including its adaptation to different soil conditions, resistance to diseases and pests, leafing time, late or early maturity, fruit yield and quality, tree growth. Previously, Northern California black walnut seedlings (*J. hindsii*) and Jugrans regia L. walnut seedlings were used as rootstocks in traditional walnut cultivation in the United States. *Juglans regia* L. is still widely used as a rootstock in some countries all around the world. Paradox (*Juglans* *hindsii* x *Juglans regia* hybrid) and Royal (*J. hindsii* x *J. nigra*) rootstocks have been used for a long time in America. Up to 80% of walnut orchards in California is home to Paradox, since it is a rootstock that grows strong and is resistant to some diseases. Paradox is a rootstock stronger, more resistant to Phytophthora species and more tolerant to soil salinity than *J. regia* (Forde 1975; McGranahan and Catlin 1987; Baumgartner et al. 2013). Disease and pest factors such as Amillaria root disease, gall mite and Phytophthora cause significant economic losses in walnut production (McGranahan and Leslie 1991). On the other hand, regarding Paradox rootstock is tolerant to blackline (CLR) disease, breeders have changed their direction to breeding rootstocks resistant to both blackline (CLR) disease and soil-borne diseases. Thereafter, at the end of these breeding studies, Vlach, RX1 and VX211 rootstocks were developed. Regarding their tolerance to Phytophthora and nematode respectively, RX1 (*J. microcarpa x J. regia* hybrid) and VX211 (J. hindsii x J. regia hybrid) rootstocks were commercially introduced (Leslie and McGranahan 2014).

Rootstock breeding studies have been carried out in China, which is one of the leading walnut producing countries in the world, and rootstocks such as Jin RS-1, Jin RS-2, Jin RS-3 have been created. It has been reported that these rootstocks have high resistance to cold, diseases and pests. It has been stated that they are suitable rootstocks especially for northern regions of China that are exposed to frost (Bernard et al. 2018; Zhang et al. 2013b). Furthermore, studies on rootstock breeding are still ongoing in countries such as Turkey and Iran regarding the criteria such as dwarfing, tolerance to salt and drought stress and resistance to soil-borne diseases such as *Agrobacterium, Phytophthora and Armillaria*.

4. GERMPLASM, BIODIVERSITY AND THEIR CONSERVATION IN WALNUT BREEDING

Like other fruit species, it requires a very long time to breed a new variety of walnut. In recent years, biotechnological developments have provided significant gains in terms of the breeding cycle and biodiversity in walnut breeding. These developments have accelerated the process and especially helped farmers gain time (Van Nocker and Gardiner, 2014). Besides, biotechnological studies both ease the process to create a gene bank consisting of superior genotypes and save a significant amount of time in the selection of the desired superior genotypes in breeding studies. As is known, conservation of biodiversity is a global problem since the lands where walnut genetic resources are located are under great threat with commercial or economic activities such as hydroelectric power plants, urbanization areas, timber trade. Therefore, the only way for conserving germplasm resources is the creation of new clonal gardens by identifying superior genotypes in the field and it is an urgent precaution in order not to lose our resources. In particular, genotypes obtained from selection studies conducted in Turkey from past to present should be protected by establishing gene centers in 3 or 4 different regions of Turkey and granting these genotypes selected in these centers. In other words, classical biodiversity conservation methods are not sufficient to solve this problem. For this reason, new biotechnological methods such as tissue culture, plant cell culture, anther culture, embryo culture are very applicable and useful new techniques in preserving gene resources. Thus, modern biotechnological applications can provide significant convenience in the protection of gene resources in the world (Ogbu 2014; Pathak and Abido 2014). Since the first walnut orchards established in the world consisted of seedlings propagated by seeds, a high degree of genetic diversity has increased in the world.

4.1. Cultivar Characterization and Phylogeny

As it is known, newly bred varieties in France and California have been an important resource in the establishment of new gardens and performing breeding studies all over the world (Germain 1999; Tulecke and McGranahan 1994). Although the French Fernor variety is preferred in cold regions, the Chandler variety is still preferred as the favorite walnut variety throughout the world. The Chandler variety was patented and marketed in 1979 by the University of California at Davis. Chandler is a hybrid of Pedro and UC 56-224 and is preferable due to its lateral bearing trait, thin shell, high walnut quality, a kernel that can be removed easily from its shell and late leafing characteristics (Vahdati, et al., 2019).

4.2. Molecular Breeding

Next-generation sequencing (NGS) techniques, bioinformatics tools, high-throughput genotyping platform, genomics-based approaches such as genome-wide association studies (GWAS), marker-assisted selection studies (MAS), genomic selection, and genomic editing using the CRISPR-Cas9 system provide conveniences and new expansions in walnut breeding. The biggest problem in walnut breeding is the high levels of heterozygosity in the long juvenile phase (Vahdati, et al., 2019). It takes at least 15-20 years to breed new varieties with classical breeding methods. For this reason, we have to try new technologies that will help us get results in a shorter time.

4.3. Molecular Markers in Fruit and Nut Breeding

Many studies in fruit breeding have described genetic differences in fruits. Although morphological markers are existed for describing these differences, there is a need for indicators to detect genetic diversity more accurately and clearly. Molecular markers are widely used in cultivar breeding to analyze genetic relationships between wild and cultivated individuals and related species, to identify cross-species hybrids, and to analyze germplasm variability.

4.4. Molecular Marker Systems

Researchers benefited greatly from isoenzyme markers in genetic studies in the past years. Isoenzymes have been commonly used in walnut breeding for interspecific hybrid identification, genetic diversity or relationship analysis, and determination of genotype origin (McGranahan et al. 1986; Cheng and Yang 1987; Aletà et al. 1990; Fornari et al. 2001; Busov et al. 2002; Ninot and Aletà et al. 2003; Vyas et al. 2003). Marker techniques such as restriction fragment length polymorphisms (RFLPs) and robust DNA markers with higher rates of polymorphism are applied in walnut breeding. Among these techniques, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) techniques are still adopted today as they are cheap and fast methods in breeding studies. These techniques are still used in walnut breeding because they provide significant convenience in the identification of walnut germplasm, evaluation of genetic diversity, and uniformity and stability of varieties (Vahdati, et al., 2019).

5. Overview of the Diversity and Fingerprinting Techniques in Walnut

The methods that have been used in walnut breeding so far can be categorized as follows;

a) Hybridization-based markers

The Restriction Fragment Length Polymorphism (RFLP) technique was the first to use isoenzyme RFLP markers to identify the origin of somatic embryos derived from ovule tissues. This method is adopted in walnut breeding for genetic mapping and determination of genetic diversity. While using this technique, large amount of pure DNA, low level of polymorphism and radioactivity complexity are also used so as to contribute to the development of various alternative technologies (Bernard et al. 2018).

b) PCR-Based Markers for Walnut Genome Profiling

In walnut breeding, Random Amplified Polymorphic DNA (RAPD) markers are widely used in genetic diversity and mapping studies including amplification of genomic DNA in the polymerase chain reaction. It is preferred by breeders due to the low cost and fast results (Welsh and McClelland 1990; Williams et al. 1990) It is possible to divide PCR-based markers used in walnut breeding into 5 groups.

1) Random Amplified Polymorphic DNA (RAPD)
2) Sequence Characterized Amplified Region (SCAR)
3) Inter-Simple Sequence Repeat (ISSR)
4) Amplified Fragment Length Polymorphism (AFLP)
5) Simple Sequence Repeat (SSR) or Microsatellite Technique

In addition to the breeding methods mentioned above, the new breeding techniques listed below are used in the walnut cultivar and rootstock breeding. In this section, the details of the techniques are not given but only their names are mentioned in the order (Vahdati, et al., 2019).

c) High-Throughput SNP Assays in Walnut
d) Nuclear Ribosomal Internal Transcribed Spacer (ITS)
e) Emerging Marker Technology: Genotyping by Sequencing (GBS)
f) Physical Mapping
g) Genetic Mapping and QTL Detection
h) Next-Generation Mapping

ı) Comparative Mapping

i) Walnut Genome Sequencing

j) Genomics-Assisted Breeding in Walnut

k) Application of Functional Genomics in Genomics-Assisted Breeding

l) Genetic Engineering

m) Mutation Breeding

n) Hybridization

CONCLUSION

In recent years, after understanding the importance and economic value of walnuts in human health, the breeding varieties with high quality have increased. In order to set an example of the dominant breeding factors in walnut breeding; the criteria includes earliness, white-colored kernels which are removed easily from the shell, high yield, lateral bearing habit, productivity, dwarfing, late flowering, tolerance to salt and drought, resistance to diseases and pests such as *Agrobacterium*, *Phytophthora*, *nematodes*, *Aceria avanensis* and *Armillaria*. Breeders use breeding methods such as germplasm assessment, selection, hybridization, genetic engineering, mutation breeding, genome sequencing, bioinformatics, marker-assisted selection, haploid and polyploid induction. In recent years, global climate change has led to significant changes in walnut production and breeding. Regarding climate change, new strategies have been recently developed in terms of walnut breeding. Accordingly, the breeding programs and methods recommended to be used are as follows.

The new walnut breeding strategies should include criteria such as low chilling requirements, early harvest and late leafing characteristics; maintaining and preserving genetic diversity for breeding new varieties and rootstocks; simultaneous use of transcriptomics, metabolomics and proteomics techniques to understand drought and salinity tolerance characteristics in walnuts; genomic selection; high-throughput phenotyping combined with genome-wide association studies in order to map genetic information associated with complex traits of walnut; production of haploid, double-haploid and wide hybrid plants to enhance genome assembly quality and focusing on the use of CRISPR-Cas9 systems in new walnut breeding studies (Vahdati et al, 2019).

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Part XI

Advances in Walnut Breeding

Assoc. Prof. Murat GUNEY[1]

Walnuts (*Juglans regia* L.) belong to the family Juglandaceae. In addition to the genus *Juglans* L., the family includes Carya Nutt. (pecans and hickories), Pterocarya Kunth. (wingnuts), Platycarya Sieb. & Zucc., Engelhardia Lesche., Alfaroa Standl., and Oremunnea Oerst. The genus Juglans consist of approximately 20 species having 32 chromosomes. Juglans is comprised of a single dichogamous monoecious species *J. regia* L. Juglans cinsi içerisinde çoğunlukla *Juglans regia*'nın kültürü ve ticareti yapılmaktadır (Woodworth, 1930); (Manning, 1978); (Şen 1986).

Juglans regia species, which has a wide natural distribution area in the temperate climate zone around the world, was taken to geographical regions outside its natural area as a result of various migrations, economic and social relations between tribes and states, and thus gained new growing areas. Due to long cultivation history of walnut, it is the most developed horticultural plant and different walnut species is widely cultivated. Today, walnut has become a type of fruit grown in many parts of the world, except in tropical and very cold

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regions (Gunn et al., 2010); (Aradhya et al., 2017). A large area of the central Asia Mountain is estimated to be the origin of *Juglans regia* and the introduction of this precious nut to European agriculture and commerce was occurred by the ancient Greeks. However, *Juglans regia* grows wildly or semi-cultivated plant in a broad area from southeastern Europe and the Caucasus to Turkey and Iran. More later, *Juglans regia* has been introduced into North America, thereafter, it has been referred to as the English walnut to be distinguished from the American black walnut (Leslie and McGranahan, 1998).

Turkey is one of the homelands of *J. regia* and has an important position in the production of walnut in world. The Anatolian region of Turkey has a wide range of climate from the mild and humid Black Sea region to the continental climate of hot dry summers and cool winters. In Turkey, walnut trees are characterized as border and shade trees in flat lands and as erosion tree on sloping lands and as a flood-preventing tree in the valleys. Although walnut trees grow across all these regions, the area of native forest is mainly limited to central, north and north-east Turkey (Davis, 1982). Akça and Şen (1994) reported that more than 10,000 walnut trees are naturally grown in Turkey.

Turkey as one of the homelands of walnut, ranks among the first producing countries of walnut (Bükücü and Sütyemez, 2016). As in many of the other countries, walnut has been produced with seeds for thousands of years in our country. (Çeri, 2021). Even today, walnut types offered to the market are far from the standard due to the cultivation of walnut from seed.

In foreign trades, markets have become more selective in quality, therefore, the demand for quality shelled-walnuts with improved quality of kernels has increased. However, this demand could not be met due to the fact that Turkey do not have a certain standard in walnut cultivation leading Turkey to be lowered in world exports rank (Sandal et al., 2018).

Due to a very long juvenile period of the walnut tree, breeding studies take many years. Therefore, there are a limited number of cultivars within this species and present cultivars have emerged both by natural selection and hybridization (Ölez, 1971); (Şen 1980); (Sütyemez and Kaşka, 2005); (Bükücü et al., 2020b); (Sütyemez et al., 2021a).

Breeding objectives

Turkey which is located in the natural distribution area of walnuts, is one of the oldest walnut-producing countries in the world. Since walnut has been propagated by seeds for thousands of years, walnut population has been formed with very different characteristics. The walnut population in Turkey is an extremely important resource for breeding programs (Pereira et al., 2007).

Despite other applications of walnut tree in industry and folk medicine, all walnut species are commonly produced for their edible kernels. However, kernels wrapped thick hard shells are dissuasive factor in commercial production, and the species with thin shells are mostly preferred for cultivation (Beer et al., 2008); (McGranahan and Leslie, 1990); (Feng et al., 2018).

Walnut is a monoecious tree in which the tree has both clusters of male (catkins) and female flowers (pistillate) in different places of the tree and are pollinated by wind (Şen 1986); (Akça and Ozongun, 2004); (Sütyemez et al., 2021b). Walnut trees also exhibit both protandry (male maturing earlier than female) and protogyny (female maturing earlier than male) (Woodworth, 1930); (Forde and Griggs, 1972). Therefore, dichogamy promotes out crossing. Wind pollination and bloom overlap are vital for walnut production (Lloyd and Webb, 1986). If the pistillate flower are pollinated successfully, the hull of walnuts separates from the shell at maturity (Manning, 1978). The male clusters containing 10-100 male flowers (catkins) are formed on lateral buds of the previous year shoots of the development period while female flowers with a number of 1-12 are formed at the ends of the current shoots (Şen, 2009).

In breeding programs of walnut, some preferred characteristics such as late leafing, flowering on the terminal and lateral branches, high fruit yield, large and relatively smooth fruit with 50% kernel, and moderate disease and pest resistance are considered for selected cultivars (Acar and Kazankaya, 2021).

Use of molecular markers

The characterization of genetic resources, population genetics, genetic mapping, and development of markers related to important traits can be studied in plants using DNA-based molecular marker techniques (Semagn et al., 2006); (Topcu et al., 2015); (Ikhsan et al., 2016); (Dang et al., 2016); (Eser et al., 2019).

Especially in perennial plants with a very long juvenile period from germination to the flowering of hybrid seed, early selection after germination of the hybrid seeds will significantly reduce the duration, cost, and workforce of the breeding programs as well as increase the effectiveness of these programs (Güney et al., 2021a, b). Besides cultivation from seed, walnut has also long juvenile period and breeding programs take a lot of time compared to other plant species (Germain, 1997); (Chen et al., 2018); (Sütyemez et al., 2021b). Therefore, molecular studies on walnuts are an indispensable part of breeding programs (Bernard et al., 2018); (Güney et al., 2021b). Because markers that can be developed related to different traits will provide early selection in breeding programs (Moose and Mumm, 2008).

Walnut varieties cultivated all over the world have emerged from chance seedlings or hybridization breeding programs. Characterization of these varieties is very important for breeders. In addition, it is very important to determine the variety's trueness-to-name in a short time in fruit cultivation. However, the morphological, physiological and biochemical methods used to characterize genetic diversity are time consuming and influenced by the environment. Contrary, molecular markers are applied for determination of genetic relationships within plant populations with higher reliability (Güney et al., 2018); (Güney et al., 2019); (Güney et al., 2021a, b). Moreover, it has been possible to characterize genetic materials from different ecologies through the development of DNA marker techniques. Walnut cultivars and genotypes found in genetic resources were identified using different molecular marker techniques such as AFLP (Bayazit et al., 2007), RAPD (Ahmed et al., 2012), ISSR (Potter et al., 2002), and SSR (Woeste et al., 2002); (Güney et al., 2021b).

In molecular breeding, molecular markers are used in conjunction with linkage maps and genomics to select plants with desirable traits based on the genetic assays (Vinod, 2006); (Güney, 2016). In order to create an ideal genetic map, first of all, a reference genetic map created with common markers is needed (Winter et al., 2000); (Güney et al., 2016); (Güney, 2016); (Kefayati et al., 2019). This can then easily lead to the development of new genetic maps in different populations; placement of important characters on link groups with QTL (Quantitative Trait Loci) analysis in these different genetic maps, allowing to identify markers for important characters and characterization and cloning of important genes. Therefore, the construction of reference genetic maps in walnut are very important for breeding programs. (Woodworth, 1930); (Fjellstrom and Parfitt, 1994); (Woeste et al., 1996); (Malvolti et al., 2001); (Luo et al., 2015);

(Zhu et al., 2015). Constructed genetic map is the preliminary step for many important downstream studies such as QTL (Kefayati et al., 2019); (Bükücü et al., 2020b).

Breeding achievement in Turkey

Walnut trees can grow naturally throughout the Turkey. All pioneer countries in walnut-growing are interested in breeding new cultivars. Therefore, numerous breeding programs in walnut-growing are done worldwide, and Turkey is one of the countries with major cross-breeding programs (Ölez, 1971); (Şen 1980); (Sütyemez and Kaşka, 2005). Owing to propagation with seed not grafting since recent years, a rich source of walnut population are available in Turkey. There may be superior varieties with high quality and productivity among this rich walnut population. Recently, high-quality and productive walnut varieties that are well adapted to environmental conditions are selected via characterization studies and grafted on walnut seedlings leading to establish dense gardens (Çeri, 2021).

In Turkey, nongrafted walnut seedlings had been used in new walnut plantations until the beginning of 1970s and commercial walnut orchards were established with grafted trees approximately 50 years ago. This has provided the opportunity to select the best genotypes from natural populations for good yield and nut quality characteristics (Orman et al., 2020); (Sütyemez et al., 2018); (Sütyemez et al., 2019); (Sütyemez et al., 2021a).

Altınova-1, Altınova-2, Bilecik, Franquette, Gültekin-1, Kaplan-86, Yalova-3, Yalova-4, Şebin, Şen-1, Şen-2, Tokat-1, Yalova-1, Yalova-2, Yavuz-1, Maraş 18, Sütyemez 1, Kaman 1, Chandler, Oğuzlar 77, Hartley, Pedro, Midland, Fernor, Fernette, Balaban, Sölöz, Yabani ceviz, Niksar 1, Akça, Diriliş, 15 Temmuz, Maraş 12, Howard, Bayrak, Adilcevaz 13, Kazankaya, Maraş 12, Chandler, Potamia Erdin, Kozdere, Zengibar, Yivlik77, Efsus 46, Bahri Koz, Tulare, Lara, Cisco, Kurtuluş 100, Bertiz Hilali, Ede, Helete Güneşi, İstiklal 100, KSÜ 46, Cerit Yıldızı and Hilal are registered varieties in Turkey and the information about these varieties are available in Republic of Turkey Ministry of Agriculture and Forestry website (Anonim, 2021a).

Future trends and conclusion

Since a large part of walnut production in Turkey is obtained from seed propagated trees, standardization in production could not be achieved (Yarılgaç, 1997); (Şen, 2009); (Muradoğlu et al., 2017). The fact that its timber is valuable has also been a factor in the prevalence of walnut cultivation in Anatolia (Sandal et al., 2018). Especially the use of old trees by cutting them in lumbering has significantly affected this gene richness in walnuts. In addition, gardening has been limitedly started with grafted walnut seedlings since the 1970s (Orman et al., 2020). Recently, It is pleasing that the production of walnut seedlings has increased with the obtained standard varieties. However, to achieve the desired level in both production and export of walnut, it is necessary to product walnut commercially in accordance with the standard cultivation technique (Şen, 2005); (Sandal et al., 2018); (Varol et al., 2020).

The main desired characteristics sought in a walnut variety selected from a breeding program are yielding, good fruit characteristics, fruiting on the lateral branches, late leafing and early harvest, as well as resistance to diseases and pests. In terms of fruit characteristics, varieties with a high yield rate, light-colored interior, thin but robust hard-shelled, high-fat and unsaturated fatty acids beside non-rough hard shell are preferred (Şen, 1980); (Yarılgaç, 1997); (Akça and Köroğlu, 2005); (Akça, 2009); (Bayazit, 2011); (Anonim, 2021b); (Arcan et al., 2021).

In a breeding program, it is necessary to wait for many years from germination of the hybrid seed to bloom and bear fruit to select desired individuals with characteristics such as fruiting on the side branches, harvest date, and fruit characteristics (Ölez, 1971); (Şen 1980); (Yarılgaç, 1997); (Sütyemez and Kaşka, 2005); (Bayazit, 2011); (Akça, 2016); (Bükücü et al., 2020b); (Sütyemez et al., 2021a, b). However, it is possible to overcome such problems in perennial fruit species only by taking advantage of the opportunities provided by biotechnology. This may be possible by utilizing molecular marker systems and molecular breeding (Güney et al., 2021a, b). Developing the DNA markers associated with important characters make it possible to select superior individuals by marker-based selection without waiting for many years in terms of these characteristics, and it will be possible to work with purpose-built materials in a shorter time, with less cost and less labor (Kefayati et al., 2019); (Bükücü et al., 2020b). This will increase the effectiveness of the variety breeding program and will enable the emergence of alternative varieties in a shorter time and with less cost according to the market conditions and consumer needs. One of the most important tools in achieving breeding varieties with better yield and fruit characteristics is the creation of accurate and high-density genetic maps.

Consequently, the starting and supporting studies on molecular breeding in a strategic product such as walnut will lead to a rapid progress in its breeding and to establish dense walnut orchards with superior varieties besides meet the quality food needs of a growing population.

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Part XII

Conventional and Molecular Breeding in Walnut

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1. INTRODUCTION

Fruits such as walnuts and walnuts are one of the oldest food sources for birds and most living creatures (Woodroof, 1967). Walnuts are classified in the subclass Rosidae, in the genus Juglans spp. (Germain et al. 1999). *Juglans regia* L. is a widely distributed monoecious tree species belonging to the order Fagales and the family Juglandaceae. This family consists of 11 genera and about 50 species, of which Carya (hickory tree), Pterocarya (wing nut tree) and Juglans are its main members (Rehder, 1947). Here are more than 20 species in the genus Juglans, and they are separated into four divisions: Trachycaryon (butternut, a single species, *J. cinerea*), Cardiocaryon (walnut), Rhysocaryon (black walnut, especially J. nigra), and Dioscaryon, comprises just *J. regia* (Manning 1978). The chromosome number of all species of the genus Juglans is $2n = 2x = 32$ and has a diploid structure (Woodworth 1930). The approximate

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genome size of the walnut is 1315 Mbp (1.35 pg; monoploid genome size (1C DNA) = 657.80 Mbp. (Sarikhani Khorami et al. 2018).

The distribution areas of *J. regia* in the world are widely found in mainly temperate areas of Europe, Asia, North and South America, Australia and New Zealand and South Africa (Aradhya et al. 2006). The Greek philosopher Theophrastus mentioned in 370 B.C. that walnuts and hazelnuts were found in the Macedonian region (Jaynes, 1969). It has been determined that Persian walnuts (*Juglans regia* L.) were found in the garden of King Solomon and there were pictures of almonds for decoration in the pictures on the candlesticks in the Temple (Moldenke and Moldenke 1952). Today it is cultivated for fruit and wood products in Asia, Europe and the Americas (Aradhya et al., 2017; Bernard et al., 2018). *J. regia* is a monoecious and wind-pollinated species. North and South America are known as distribution centers of *J. regia* (Aradhya et al., 2007). It is the just species in the genus *Juglans regia* L. Juglans, which is widely cultivated in Europe. Turkey is one of the origins of *Juglans regia* L (Fao, 2021). Anatolian walnut, which has a large natural spreading area in the world, has been taken to different places by various migrations and trade caravans, and today it can be grown almost all over the world, except for the tropics. Walnuts are wind-pollinated and have monoecious characteristics, that is, male and female inflorescences are on the same tree, but in different places (Sen 1985).

Walnuts are one of the healthiest hard-shelled fruits. As such, we can consider walnuts as foods enriched with many nutrients that have health benefits. It is also one of the foods with the lowest glycemic index (Şen, 2017). Generally in walnuts; It is 3.5% water, 15-30% protein, 55-77% oil, 1.5-3% ash. It is rich in mineral substances such as Ca, P, Mg, Fe, Na, K. It also contains vitamins A, B1, B2, B6, C (Bayazıt and Sümbül, 2012). Benefits of walnut ın human health due to the effect of omega 3 fatty acids It has been determined that it reduces the risk of coronary heart disease, reduces triglyceride and cholesterol levels, prevents blood clotting, prevents the development of inflammatory diseases (Banel and Hu 2009). Due to the high content of silver and selenium, it has a positive effect on intelligence. Walnuts can be consumed in many different ways. It is used as a snack, in the pastry and biscuit industry, in the perfume industry, in the production of jam, halva, paint, tannin, in the plastic and rubber industry, as oil, in the pharmaceutical industry, in the production of sausage and fruit pulp (Sütyemez, 2014). *Juglans regia* is the only species whose fruit is grown for consumption. Diversity within this species is low and varieties are mostly from natural selection (Şen, 2017).

Although the high economic worth of the walnut and its deep-rooted history, reproduction is limited, especially in regions that are the gene center of the product, compared to other warm regions. The first walnut breeding plans rely on history the middle of the twentieth century. (Bernard et al. 2018). The genetic development of the walnut was previously carried out by selecting individuals with superior characteristics from natural populations. The main goal is to increase the yield and quality (large fruit, high kernel weight, light colored kernel) in walnuts, as in other fruit types.

With the acceleration of developments in plant breeding, food outturn was also positively affected and played an important role in human nutrition (Tester and Langridge 2010). This has also reduced variability in agricultural crops around the world and contributed to genetic makeup against adverse environmental conditions (Keneni et al. 2012). It is therefore significant to recognize the effects of modern plant breeding on genetic variation. At the same time, the skillful command of this genetic variation will provide valuable assistance to producers. In walnut production, adverse environmental conditions (such as drought, salt and spring frosts) negatively affect walnut yield in many regions(Cochard et al., 2002; Lotfi et al., 2009a, b; Khodadadi et al., 2016; Jinagool et al., 2018; Knipfer et al., 2018; Liu et al., 2019). One of the very significant environmental factors limiting walnut productivity in arid and semi-arid regions where walnuts are grown is thirst (Aleta et al., 2009; Vahdati et al., 2009; Famula et al., 2019). In addition, increased drought due to global climate change increases severe drought circumstances in walnut-growing regions (Lotfi et al., 2010; Karimi et al., 2018). Drought, especially during the growing period, can seriously affect walnut development and become one of the most important factors restricts product (Vahdati et al., 2009; Liu et al., 2019). Another important factor affecting the yield in walnuts is that dichogamy is very common. The fact that male and female flowers open and become active at different times is a factor that negatively affects pollination-fertilization success and reduces yield (Sütyemez, 2014).

Walnut has been studied a lot all over the world because it is a valuable plant in terms of both economic value and nutrition. With the rapidly developing technology, traditional breeding studies have begun to give way to molecular breeding studies. In this section, traditional and molecular breeding methods, advantages and disadvantages of walnut will be explained.

2. CONVENTIONAL BREEDING IN WALNUT

For an economical and standard production, the products obtained from seeds, that is, the trees grown from seeds, cause very important time and economic losses due to quality, yield and standard differences. In order to eliminate this negative situation of the walnut potential, walnut trees grown from seed should be removed and gardens should be established with grafted saplings of certain varieties. In addition to a very important time loss, this will cause a loss of yield (production) with the removal of many yielding trees. Plants that occur in propagation with seeds do not carry almost any characteristic of the mother plant (they show genetic variation). For this reason, seed propagation in walnuts is not a preferred propagation method, except for plants obtained by rootstock or seed and breeding studies (Sütyemez, 2014)

The beginning of agriculture dates back about 10,000 years, and since this time people have begun to adapt plants for their own purposes. In addition, naturally occurring beneficial traits were often transferred to different plants by human hands, as opposed to natural selection (Evenson end Gollin 2003). Genetic variation is studied from several aspects like molecular, phenotype and biochemical traits (Govindaraj et al., 2015; Guney et al., 2021a). Genetic variation in walnuts is determined by using phenotypic and biochemical prosses by determining the information in the gene and selecting superior genotypes with covetable characteristics (Bhat and Kudesia, 2011, Guney et al., 2021b).

Genetic variation is generally determined using morphological markers. Germplasm characterization and identification of varieties as the first step in genetic research, breeding programs and selection studies form the basis (Smith and Smith 1989). Toward this end, the International Union for the Conservation of New Plant Varieties (UPOV) determines principles for defining the diversity, purity, and constancy in the establishment of novel cultivars for most plants. Therefore, morphological research guides the selection of plants suitable for certain growing situations (Solar and Stampar 2011). In *J. regia*, first morphological research are related to the identification of the association between fruits with different plant characteristics (Sholokhov 1974; Komanich 1980; Şen 1985; Sharma 1996). Although there was an inverse relationship between yield and dichogamy (Kornienko 1974; Majacka 1971), no distinction could be determined between yield and fruit quality and protogynous and protandry characteristics. (Akça and Sen 1997). In a subsequent study, a correlation was found between fruit internal weight and flowering shape (Eskandari et al. 2005). Additionally, Amiri et al.(2010) determined that there is a powerful correlation among lateral branch yield and fruit yield in Iranian cultivars. On the other hand, in a study conducted in Slovenia, walnuts with high minor yield were found to be more sensitive to blight and cold (Solar et al. 2001) and sensitive to walnut blight was found to be associated with the date of defoliation (Abedi and Parvaneh 2016). Also, a negative relationship was noted between yield and tree height by Forde and McGranahan (1996), and while there was a positive correlation across altitude and frost resistance, a negative correlation was found between altitude and yield (Atefi, 1990). In countries such as Iran, it has been determined that morphological studies and selection studies in natural populations will help breeding programs (Ghasemi et al. 2012). The characteristics of these natural populations inclusive genotypes, leafing date, side branch yield, dichogamy were determined phenologically and phonologically (Ghasemi et al. 2012; Ebrahimi et al. 2015), bud burst (Arzani et al. 2008), tolerance to late spring frosts (Mahmoodi et al. 2016), fruit yield, fruit maturity, and fruit features (Haghjooyan et al. 2005; Arzani et al. 2008; Ahandani et al. 2014; Khadivi-Khub et al. 2015). In addition, the vigor of dwarf seedlings to increase planting frequency in Iran was also investigated (Rezaee et al. 2009). It was determined that there was a favorable correlation across the parameters such as seedling diameter and seedling size, number of nodes and seedlings and length of internodes, proposing that measuring of seedling height alone is enough for recognition of dwarf genotypes, also, parallel researchs were applied in Turkey (Bayazit 2012). Excellent genotypes were chosen for yield, nut features, and cold resistance (Aslantaş, 2006) and other hopeful genotypes were chosen for late flowering, walnut blight and anthracnose resistant (Yarilgac et al. 2001; Asma 2012; Fikret Balta et al. 2007; Karadag and Akça 2011). Assessment of pomological and phenological characteristics of natural populations in the Dibra area in Albania determined a high variability in fruit weight (Zeneli et al. 2005). Walnut varieties have been assessment in many such studies in Serbia (Miletic et al. 2003) and additionally some foreign variety in the climatic circumstances of South Bulgaria (Gandev and Dzhuvinov 2015). In the study conducted in India, genetic and geographical diversity were examined using 15 fruits (Sharma and Sharma 2001). In a research conducted in a garden created from seeds under the same conditions, it was determined that the population created with the genotypes selected in Italy to conserve genetic resources had as much mutable as the variation in a larger population (Ducci et al. 2010). In France, 2450 hybrids from 22 crosses across French and California cultivars were sourched. The leafing dates of the same trees at the age of 1 and a few years later were examined and showed that the feature related to the leafing date can be determined when the plants are at an early age (Germain 1990). In another study, proportion of apomixis in walnuts was examined and differences were determined between varieties grown in many European and Mediterranean (Asadian and Pieber 2005).

The Šeinovo cultivar and the Rasna selection were used as the results of the examination of 20 walnut genotypes selected from Montenegro over a three-year period. According to the international walnut descriptor, the most significant biological and pomological features were examined. Basic criteria in selection studies: late vegetation onset, early vegetation end date, kernel internal ratio, easy separation of the fruit from the skin, peel roughness, full kernel yield, delicious fruit, light colored kernel and chemical composition of the fruit. It has been determined that walnut has a successful adaptation in this region and is well adapted to the agro-ecological conditions in this region (Jacimovic et al. 2020).

2.1. Mistakes Made in Conventional Walnut Breeding

Traditional breeding practices are basically based on selection of individuals with superior characteristics, namely sectioning. However, it should not be forgotten that traditional breeding practices can be done correctly and that these selected individuals will contribute to modern breeding programs**.** Errors made in traditional breeding practices should be well known and applied correctly (Sütyemez, 2000; Şen,1985) The main mistakes made are:

- Establishment of new gardens in unsuitable climate and soil conditions
- Using plants that are healthy and not true to their name,

- Producers who do not have the necessary knowledge in terms of fertilization biology, use more or less pollinator varieties or plant wrong pollinator varieties.
- Failure to plan the trees to be planted during planting,
- Not being done in accordance with the planting technique.
- Not choosing a pollinator based on flowering characteristics of the main variety

Walnut growers have long set up new orchards with the seeds of the fruits they have selected, resulting in high genetic variety in walnuts around the world. Following improvement of inoculation techniques and walnut breeding in France and California, walnut variety were presented. Also, outside of France and California, new walnut cultivars have been presented using local genetic variation and hybridization in other countries as well. Some French/Californian cultivars are produced global. Franquette is the first of these varieties(Germain 1999; Tulecke and McGranahan 1994**).**

Due to the fact that it has a long youth sterility, the reproduction and fruiting period of walnuts is long. For the use of walnuts in breeding, their varieties must be known. On the other hand, the morphological, biochemical and physiological practies applied in the identification of varieties are methods that require a long time and are affected with environmental conditions (Topçu et al. 2015). All these features are affected by three situations: genetic substructure, environment, and interplay across the two; it is of highest significance to explained the amount of add of each of these ingredients.

3. MODERN BREEDİNG İN WALNUT

The products obtained from breeding programs are highly dependent on the existence of genetic variation, either spontaneously or by external intervention. In order to increase and maintain food production, plant breeders and geneticists are constantly working to develop new strategies and to create a food source with plants adapted to infertile soils and tolerant to adverse environmental conditions. In conventional breeding, the transfer of genetic information from one species to another is done by backcrossing over several generations. (Al-Khayri et al. 2019). With the improvement of molecular tools along with new technologies, it has become easier to determine whether a plant has a trait by using molecular markers, and the breeding period has been shortened (Arzani et al.,2008; van Nocker and Gardiner, 2014). With the sustained development of plant biotechnology, genomic information and molecular markers. They have become excellent tools in the formation, analysis and identification of genetic change for the improving of new varieties (Bernard et al. 2018).

The genetic development of walnuts began with the selection of superior trees in their main origins. Selection and grafting methods, among the methods used in traditional breeding studies, first started in France (Vahdati et al. 2019). The first walnut breeding program using hybrid plants began in the USA in 1948 with the introduction of french varieties and using local genotypes selected from seeds obtained from centers of origin (Iran, Afghanistan, China). Currently, both conventional methods with phenotypic evaluation and molecular initiatives are employ in breeding studies conducted in the USA, France, China, Iran, Spain and Italy. With the recent developments in biotechnology and genomics and variety development studies, breeding programs are also accelerating. Together with these developments, the research, identification and protection of biological diversity will enable the creation of a gene bank with the desired characteristics and the more accurate and faster work in the future by biotechnological means (Khorami et al., 2018).

4. MOLECULAR BREEDİNG İN WALNUT

The latest technologies used in breeding studies in plants using biotechnological methods: next generation sequencing (NGS) techniques, bioinformatics tools, high-throughput genotyping platforms and genome-wide association studies (GWAS), marker assisted selection (MAS), genomic selection (GS), and CRISPR- Cas9 ıt includes genome editing with the system. We explain the application of molecular breeding and biotechnological methods used in walnut breeding (Iwata et al. 2016; Laurens et al. 2018).

It is the long-lasting juvenile phase and highly heterozygous nature that affects the genetic development of walnuts In order to cope with the problems in traditional breeding methods, the use of new genomic technologies in molecular breeding methods in walnut has gained importance. Molecular marker systems used in walnut breeding and all nuclear and chloroplast genome sequence information, QTL mapping, GWAS and GS, MAS studies will all be explained under the name of molecular breeding methods.

4.1. Markers in Walnut Breeding

With the development of enzyme-based markers through isoenzyme electrophoresis, a major breakthrough in the late 1980s, the weak genetic information in plants has begun to come to light (Hunter and Markert 1957). Isozyme markers were widely used molecular markers in genetic studies until PCR-based markers were developed. Isozyme markers have different uses in walnut genetic analysis, such as identification of cross-species, genetic diversity, and determination of the evolutionary process of the genotype (Aletà et al. 1990; Arulsekar et al. 1986; Busov et al. 2002; Cheng and Yang 1987; Fornari et al. 2001; Malvolti et al. 1993, 1994; McGranahan et al. 1986; Ninot and Aletà 2003; Solar et al. 1994; Vyas et al. 2003).

The isozyme gene phosphoglucomutase found in *J. regia* was first identified and characterized by Arulsekar et al. (1986). In subsequent studies, hybrids make between Pterocarya spp and *J. regia* were defined by isozyme differenties (McGranahan et al. 1986) and the source of varied walnut types in China and Tibet was investigated (Cheng and Yang 1987). Malvolti et al. (1993) also studied genetic diversity in *J. regia* populations in Italy and In a study comparing pollen enzymes and leaves in Slovenia, it was determined that there was more variability in pollen enzymes than in leaves (Solar et al. 1994). Furthermore, a study of leaf and fruit morphology and isozyme variation in populations of *J. regia* from central Italy demonstrated an special relation among genotype and leaf morphology and among leaf morphology and fruit morphology. In these two studies, it determines that there is a causal relationship between genotype and phenotype (Malvolti et al. 1994). In many different studies, isozymes were utilize to determine genetic variation / correlation in *J. regia* / *J. nigra* analysis (Fornari et al. 2001; Ninot and Aletà 2003; Busov et al. 2002), or for cultivar determination (Aletà et al. 1990; Vyas et al. 2003).

4.2. Hybridization-Based Markers

4.2.1. Restriction Fragment Length Polymorphism (RFLP)

Molecular identification methods create a precious and effective technique to scan germplasm collections and ease the utilization of these source by breeders and investigators (Mason et al. 2015). Two methods was used to define the source of the somatic embryos of the ovule in the *J. regia* (isosyme and RFLP) (Botstein et al. 1980; Aly et al. 1992). Parentage testing was performed quickly on plants propagated in vitro. Leter, a California group carried out three studies in 1994 utilizating with RFLPs. First utilization, the double-group method with arithmetic mean (UPGMA) cluster analysis (Nei, 1972) describe that the California germplasm was interrelated that of France and Iran and had less resemblance to Nepal, China, and Japan (Fjellstrom et al. 1994). [*J. Hindsii* × *J. regia*] × *J. regia* was performed to interspecies backcross analysis, generating the first linkage map of 12 linkage groups (LGs) on 1660 cM in length.

(Fjellstrom and Parfitt 1994a). The taxonomic correlation between varied Juglans species were studied (Fjellstrom and Parfitt 1994b) and it was determined that J. cinerea does not belong to the Cardiocaryon division, as stated in the literature, but should belong to the Trachycaryon division (Manning 1978).

The biggest disadvantages of the RFLP method are the low rate of polymorphism, the difficulty of using it (Wani et al. 2010), deficiency of automation and the need for the more quantit of pure DNA (Schlötterer 2004). Therefore, novel system rely on polymerase chain reaction (PCR) were advanced.

4.3. PCR-Based Markers for Walnut

4.3.1. Random Amplified Polymorphic DNA (RAPD)

A working group in California found RAPD' markers using a population of hybrids. [*J. hindsii* × *J. regia*] × *J. regia* and a revised walnut genetic map including previously published (by Fjellstrom and Parfitt, 1994a) RFLP markers. This study determined the number of LG groups as 15, not 12, but a total of 16 LG groups, which should be in walnut, could not be established (Woeste et al. 1996b). Other studies by the same team defined and characterized a RAPD marker related to a hypersensitivity reply to cherry leaf curl virus, black streak disease in walnuts. (Woeste et al. 1996a). In other walnut hybrid population {[*J. hindsii* × *J. regia*] × *J. regia*} during the transfer of genetic information to hybrid plants, a little correlation was determined among genetic and morphological interval (Woeste et al.

1998), Morfolojiye dayalı seleksiyon, bir türden diğerine genetik bilginin aktarılması sırasında ebeveyn genomunun korunmasında etkili bir yöntem olmadığı ileri sürülmüştür. RAPD markers have also been used in plants used in a breeding program at the University of California, including BPayne, BMeylan, and BFranquette, to study genetic relationships between *J. regia* cultivars. (Nicese et al. 1998).

4.3.2. Sequence Characterized Amplified Region (SCAR)

RAPD markers are less reliable in plant breeding due to their low repeatable, but they are simple to improve and easy to use. However, it has the possibility to convert RAPD amplicons into sequence-characterized amplified region (SCAR) markers to improve their reusability. Several recent research have defined molecular markers related to economically significant features in walnuts, inclusive a RAPD marker associated with earliness (Keqiang et al. 2002), a SCAR marker linked to hell thickness (Li et al. 2007) and a SCAR marker associated with earliness (Li et al. 2010).

4.3.3. Inter-Simple Sequence Repeat (ISSR)

ISSRs are markers with a single long oligonucleotide primer stable at both at the tip with a simple sequence repeat (Godwin et al. 1997). ISSR markers have used in walnut genetic diversity experiments, phylogenetic analyzes and many other genetic experiments (Christopoulos et al. 2010; Ji et al. 2014; Pollegioni et al. 2003; Potter et al. 2002), genetic mapping creating (Malvolti et al. 2001) and examination to define the provenance of a genotype (Malvolti et al.

2010). Altogether, the RAPD and ISSR markers have been frequently employed by scientists working in walnuts for the description of the regional *Juglans regia* germplasm from Turkish, Iranian, Romanian, Greek, and Chinese. (Fatahi et al. 2010; Christopoulos et al. 2010; Pop et al. 2010; Qianwen et al. 2010; Erturk and Dalkilic 2011; Ji et al. 2014;).

4.3.4. Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism markers (AFLPs) in walnut (Vos et al., 1995) have been applied for different purposes in many genetic studies**.** AFLP markers, like RAPDs, are often sequenced using fragments to create SCAR markers (Lecouls et al. 2004). AFLPs were search to examine the genetic diversity of Xanthomonas arboricola pv juglandis, most significant diseases of *J. regia* reason walnut blight (Loreti et al. 2001). In this study, 66 isolates of *X. arboricola* pv juglandis from varied countries were describe. The same AFLP markers were application to determine the varied in host-associated carob moth (*Ectomyelois ceratoniae*) populations in pomegranates, pistachio and walnuts in Iran (Mozaffarian et al. 2008). Sütyemez (2006) used AFLP markers for the first time to study genetic variation in walnuts. AFLP markers are used to determine superior genotypes in walnut genetic studies (Kafkas et al. 2005), to reveal genetic variation (Ali et al. 2016; Bayazıt et al. 2007; Qing Guo et al. 2010), to determine the evolutionary process (Wang et al. 2010) and for the identification of walnut variety (He et al. 2010; Ma et al. 2011; Xu et al. 2012). A group of researchers used AFLP markers to identify genetic correlations in wild *J. regia* and *J. sigillata* populations of economic value from Sichuan province in China (Chen et al. 2008, 2009).

4.3.5. Simple Sequence Repeat (SSR) or Microsatellite

Known simple sequence repeats (SSRs, Microsatellites) (Morgante and Olivieri 1993) are much stronger and enlightening markers for fingerprinting analysis and determination of genetic variety, genetic mapping, population structure and marker assisted selection (MAS) in plants due to their abundance, dominance and high repetitivable and high polymorphism in the genome (Wani et al. 2010). SSRs have the very commonly utilization markers for marker assisted breeding for fruit trees and crop improvement in many plants over for 10 years (Bernard et al. 2018; Singh et al. 2008).

Due to the unique properties of SSR markers, walnuts have become the preferred markers for many utilization in genetic research and breeding. In walnut cultivation, SSRs are employed to assess the genetic variety of the resulting property before selecting parent property that will aid maximal this variation for subsequently ideal integrations. SSRs have been used in walnut breeding genetic studies for many diverse goals: Fingerprint clones with quality timber in Juglans nigra (Woeste et al. 2002), determination of genetic variety (Gunn et al. 2010; Victory et al. 2006; Wang et al. 2008 Pop et al. 2013) F1 recognition (Pollegioni et al. 2008) and parental recognition (Pollegioni et al. 2009; Robichaud et al. 2006).

Genetic variety was determined in *J. regia* and J. sigillata populations using SSR primers developed from J. nigra, and the UPGMA dendrogram analysis indicated a different geographic trend from the known taxonomic sorting (Wang et al. 2008). In various studies that studied the genetic variation of *J. regia* and *J. sigillata* in Yunnan, China, using SSR markers, humans effects and role in biodiversity of walnut were investigated (Gunn et al. 2010). These markers determined that the two species were not distinguishable, and the researchers reached two different conclusions: that *J. regia* and J. *sigillata* could be new species or different species in the gene flow (Qi et al. 2011).

Other Juglans species, like native populations of J. mandshurica in northern and northeastern China, were examined using SSRs (Bai et al. 2010). Results, comprising cpDNA fragments, determined that two unrelated sites were preserved in northern China the last ice age, in contrast to many another moderate forest trees that were taken to southern China. Novel ESTs from *J. regia* were studied in *J. nigra* and Carya spp strains and the high polymorphism display of these EST markers was found to be transferable across the two strains. (Zhang et al. 2013). In conclusion, naturally occurring individuals of J. nigra, J. major and J. microcarpa in Texas were examined using SSR markers and it was determined that interspecies hybridity happened in these trees (Grauke et al. 2012). In *J. regia*, an significant Italian variety, Sorrento, DNA fingerprinting analyzes were determined using SSRs (Foroni et al. 2005, 2007), In addition to different Italian walnut sources, SSR markers were employ to determine and identify other varieties obtained by selection studies (Pollegioni et al. 2011). In subsequent studies, SSR markers were employed to examinate genetic differences using Iranian genotypes (Karimi et al. 2010; Mohsenipoor et al. 2010; Ebrahimi et al. 2011; Mahmoodi et al. 2013; Najafi et al. 2014). Spanish and US cultivars were determined to differ from California cultivars using SSR markers (Ruiz- Garcia et al. 2011). In addition to examinate utilization SSRs, differences and genetic correlations between Indian genotypes have been investigated (Noor Shah et al. 2016), between trees from cold fields of the USA and Europe(Ebrahimi et al. 2017), and between genetic resource in China (Han et al. 2016; Wang et al. 2016) and Romania (Pop et al. 2013).

The recent years, three recent papers have been published. First, a high-throughput multiplex PCR protocol was developed using 11 SSR markers improved in J. nigra to examine the differences in Croatian genetic sources(Ćelepirović et al. 2016). Another study concerned populations of J. *regia*s naturally growing in the Italian Alps with genetic variation (Vischi et al. 2017). Bacterial artificial chromosome (BAC) end sequences in *J. regia* were employed to develop novel SSR markers (Wu et al. 2012; Ikhsan et al. 2016), SSR markers have been developed to increase the number of specific alleles from genomic DNA libraries with repeat regions like 'BAAC' or 'BAAG'(Topçu et al. 2015), and EST sequences for the transcriptomes of different tissue samples (Dang et al. 2016). Polymorphic SSR markers were developed from Class I repeats from Chandler's DNA sequences. Of the 16 ceviche varieties (8 Turkish, 3 French and 5 US), 800 SSRs were designed and tested, 88 (11%) non-band producing, 161 (20.1%) monomorphic and 551 (68.9%) polymorphic (Eser et al. 2018).

The phenological and morphological properties of the material "Esterhazy II" in the Hungarian gene center were compared with those of "Milotai 10" and "Chandler". Viewed characters; budding, flowering time, variety of polygamy, ripening time, kernel and nut characteristics. SSRs were used in fingerprint analysis to identify the same varieties and to determine the association of the analyzed “Esterhazy II” genotype with different Hungarian cultivars. As a result, it was determined that the genotype known as "Esterhazy II" could be observed in several different cultivars (Bujdoso et al. 2021). All these used markers could potentially be utilization for germplasm characterization and generation of connectivity maps in walnuts

4.4. High-Throughput SNP Assays in Walnut

Single nucleotide polymorphisms (SNPs) (Wang et al., 1998) have many superiors over previously found markers because they are Genotyping by Sequencing (GBS) markers for automated high-throughput analysis in the genome in large quantities and currently at moderate cost **(**Schlötterer 2004). The first utilize of SNP markers to identify *J. regia* variants (Ciarmiello et al., 2011) used the 5.8S coding region of rDNA-repeat (ribosomal DNA) the operon segment containing and duplicated spacer regions (ITS1 and ITS2) the first and second. 244 SNPs and one short insertion-deletion (InDel) were obtained by aligning the sequences. It was determined that these regions could be used in phylogenetic analyzes to characterize of *J. regia*. It also allowed the development of a genome-wide infrastructure from coded sequences to discover SNPs. 22.799 SNPs were discovered at Chandler and 6000 SNPs were selected to determine the genetic makeup of a mapping population (You et al. 2012). Subsequently, 48,165 SNPs and 1037 InDels were identfied in *J. regia* by local genome pyrosequencing (Liao et al. 2014). It has been stated that these SNP markers will be of great benefit for genetic variation analysis, determination of nucleotide differences in the genome, formation of genetic maps and another breeding practices like marker assisted selection. The causative agent of thousand cankers disease in the USA, Geosmithia morbida, was also examined utilizing SNP markers of 209 isolates in a population genetic research (Zerillo et al. 2014). Eventually, a study with SNPs evaluated syntenic across walnut and some other plants. These plants contained three long-lived perennials (Vitis vinifera, Populus trichocarpa, and Malus domestica) and three short-lived plants (Cucumis sativus, Medicago truncatula, and Fragaria vesca)**.** As a result, the walnut genome was found to be closer to the genome of three of the woody perennial plants (Luo et al. 2015).

Recent progresses in next-generation sequencing tools and the sustained reduce in expense have conculuded in the huge lineage of sequence information sets, leting the fast exploration of single nucleotide polymorphisms (SNPs). SNPs deliver along the genome and are extencively employed in animal and human genetic study, however their utilization in plants, and especially in walnut, is in the early phases. So far, the last presence of genomic and transcriptomic databases has accomplish feasible the exploration of SNPs in silico, utilization bioinformatics mediums. A walnut genome sequence (cv. Chandler v1.0) has recently been discovered (Martínez-García et al. 2016; presenting at https://www.hardwoodgenomics.org/english-walnut-genome), 27 genomes of most significant constitutive in the walnut recovery program at the UC Davis have been resequenced and,at last, a new Axiom® Walnut700K SNP array has been planned by UC Davis for walnut changeability, linkage mapping and related mapping analysis (Marrano et al. 2019; Neale et al. 2017). The attendance of a high-density genotyping sequence in walnut lights novel chances to perform GWAS and genomic choice in walnut-breeding performs and different walnut populations (Bernard et al. 2018; Marrano et al. 2019; Neale et al. 2017).

4.5. Physical Mapping in Walnut

The physical map demonstrates the physical interval across loci. The copy of exogenous DNA in bacterial artificial chromosomes (BACs) is a novel process for genome investigation (Choi and Wing 2000). To create physical map in walnut, it was amplified and isolated in vitro from Persian walnut (Chandler), and bacterial artificial chromosome (BAC) two libraries were generated from the isolated DNA and digested with HindIII or MboI endonucleases. Bases of 135 kb and 120 kb in length were added to the DNAs cut with HindIII and MboI endonucleases, respectively. A whole of 129,024 copies, 64,512 per BAC library, were prepared at 336 plates (Wu et al. 2012). Presuming walnut genome length of near 606 Mb, the two generated BAC libraries expressed almost 27x genome equal. In research, fluorescence-based upon BAC identifying individuals and BAC-end sequencing were applied utilization a high-throughput BAC DNA identifying individuals technique (Luo et al. 2003). A total of 52,840 BAC copies off the (Chandler) HindIII and MboI libraries from Iranian walnut were sequenced and 48,218 walnut BESs were uploaded to GenBank (Wu et al. 2012).

As a result of Blast2GO, 1330 matchless GO terms were identified in 6396 BESs. Dispersion of GO terms in the class of biological way, molecular process, and cellular ingredient indicated that the walnut genes contain a wide field of functional sections and biological transaction. Thanks to putting in order of BES with ESTs and all walnut genome shotgun sequences. Nearly 4000 SNPs were explored and to create genetic mapp in individuals of cvs. Chandler × Idaho, which finally caused anchoring of BAC contigs upon a linkage map. Physical mapping in walnut yielded two markers containing the LB1 locus related to the lateral bed. Lately, a 2-year program at the University of California, Davis was begun to explore the initiative mutation at the LB1 loci, improve an estimated SNP marker, and apply the marker in walnut genetic recovery. Over the first stage of this program, on 700 progenies off an inbred cv Chandler population were defined that bear recombination in the LB1 area. Front outcomes from this research put forward three LB1 nominee gene (Dvorak et al. 2015).

The study was applied to appraise 25 excellent walnut varieties in terms of phenotypic and cytological features. To this end, 560 walnut varieties in southwest Iran were examined according to UPOV and IPGRI descriptor. Later a 2-year first evaluation, 25 excellent genotypes were chosen for phenotypic and genome dimensions evaluated. Flow cytometry was utilized to evaluation the genome size of the chosen excellent varieties. High genetic variety existed in the walnut population gathered off the southwest of Iran. The chosen excellent cultivars had very yielded high lateral branch yield, shell thickness, high kernel ratio and seed weight, and a light interior color that could be easily separated from the Shell. Therefore, genome size can be take into account as a powerful and precious tool to forecast nut size and kernel and nut weight (Sarikhani Khorami et al. 2018).

4.6. Genetic Maps and QTL in Walnut

Genetic map: the basis of distinction of markers in the course of meiosis, indicating the location and relative genetic intervals among markers throughout the chromosomes occurs chromosome recombination (Paterson 1996). Locating genes or QTLs related to relevant traits in genetic maps is the first step. Genetic maps are important for breeding studies and are of great benefit for marker-assisted selection (MAS) (Semagn et al. 2006). Intraspecies and interspecies populations were established to set up genetic maps and to identify QTL regions in walnuts.

The initial genetic map in walnut was composed with the utilization of RFLP markers. Offspring of 63 individuals from a Next-Generation Mapping between different species backcross of [J. hindsii × *J. regia*] ×*J. regia* was examined. These thriving in a J. regia Hartley orchard open-pollinated male-sterile *J. hindsii* × *J. regia* trees were formed from backcross plant (Paradox Mom) Forty-two RFLP markers were located in the 12 LG groups, and the genome size was predicted at 1660 cM, inclusive the contain of the walnut genetic map with 66 RAPDs. (Fjellstrom and Parfitt 1994a). A new and more advanced genetic map was created that includes these RAPDs and previously identified RFLP markers. This new map consists of 15 LG groups and one hundred and seven markers (Woeste et al. 1996b). The inbreeding cross of *J. regia* was investigate with both RAPDs and isozymes. A population of 81 F1s from the *J. regia* BLara 480^ and *J. regia* Chandler 1036^ cross was used with 120 RAPDs and four isozymes. (Malvolti et al. 2001). Parental maps were composed with LG groups 11 in the female map and 10 in the male map.

Due to the lack of existing information in the walnut literature, it was targeted to create the first SSR-based genetic linkage map with the 'Chandler' × 'Kaplan 86' F1 crosses and to determine the QTL for leafing time in walnuts. Of the 1437 SSR primer pairs, 386 SSR markers were located in 16 LGs. The overall length of the consensus map was 1568.2 cM, with a mean length of 98.0 cM. The Chandler linkage map contained 279 SSR markers with a total length of 1285.8 cM, while the Kaplan-86 genetic map included 273 SSRs with a total length of 1574.4 cM. As a result, it was published as a linkage map that was created based on the SSR marker, with medium density and could be considered as a basis for genetic and molecular investigations in J.regia and other species. A QTL region was determined for foliation time, which express 52.0-68.8% of the phenotypic diversity in both parental and consensus maps. It has been stated that this defined QTL region can be used in walnut breeding studies in the coming (Kefayati et al. 2019).

4.7. Next-generation mapping

A 6K SNP Select Infinium BeadChip was created (You et al. 2012) and utilizated, in the last four years, to genotype an F1 offspring of 425 plants from an intraspecific cross between ‘Chandler’ and ‘Idaho’ (*J. regia*) Number of all SNP markers placed on the map 1525 SNP in 16 LGs and the length of the genetic map was determined as 1049.5 cM (Luo et al. 2015). The available genetic map was employed to establish a walnut physical map by 15,203 exonic BAC data. The physical map is near to the approximative length of genome, 606 Mb, by a predicted total size of 736.1 Mb and symbolists the general of the genome. (Luo et al. 2015). From the mapping study, two markers were also found to locate the LB1 gene including in lateral bearing phenotype on LG11. (Dvorak et al. 2015). As a result, an F1 population of 84 individuals was generated with an in-species cross across ‘Yuan Lin’ and ‘BQing Lin’ (*J. regia*). 2577 specific locus amplified fragment (SLAF) markers were made use of in three types (just 2300 SNPs (89.25%), 87 InDels, 190 SNPs and InDel). These markers were located on 16 LGs and the size of the resulting map was determined as 2457.82 cM. (Zhu et al. 2015). In addition, a QTL region related to anthracnose (Colletotrichum gloeosporioides) resistance, which is also a significant trait in Chinese plant varieties, was determined in LG14. QTLs with 10 markers between 165.51 and 176.33 cM and LODs between 3.22 and 4.04 were determined in LG14. Utilizing 14 polymorphic SSR markers, genetic structure and distinction were appraised in the Iranian walnut germplasm collection consisting of 204 individuals from the USDA germplasm collection and a total of 399 individuals from 62 elite germplasms often utilized in the walnut breeding program at UC Davis. This study supplies precious knowledge on genetic variety and applies to resequence to determine SNPs in the walnut genome and be assisted to define diverse genotypes in these populations. In addition, molecular variance (AMOVA) analysis disclosed ~87% of the diversity into populations, by just 13% of important genetic change take into account for diversity across groups (Dvorak et al. 2008).

In another study, single nucleotide polymorphisms (SNPs) were developed with a comparison of diverse resources of data knowledge; BAC end sequences, SOLiD shotgun genome data and RNAseq from different walnut materials. These collations detected ~6000 SNPs that were operates by Illumina to create an Infinium array. This SNPs were employed to compose the genetic map with examining 352 offspring off a cv. Chandler × cv. Idaho progeny and these map have been utilized to sequence any phenotypic feature to linkage groups (Dvorak et al. 2011). Lateral bedding in walnut genetic breeding is one of the main determinants of output and one of the most significant targets in breeding. Martínez-García et al. (2014) in a project to develop a high-throughput genotyping platform for lateral transport (KASPTM genotyping) used molecular markers developed by Dvorak et al. (2011). Allele-specific PCR (KASP) genotyping method was employed in the research owing to its capability in genotypic thousands of plants utilizing a little series markers with cheap and loud correctness.

A genome-wide association study (GWAS) was made to identify markers associated with leaf budding time and flowering characters. The data of 13 distinct characters were recorded serial with 3 years of data. The relationship between the variation in 188 walnut In the study, using the DART-seq method for investigating the genetic constitution with 14,761 SNP and 18,758 DArt markers, the determination of the markers related to these characters was studied. In conclusion, each of the 16 QTL regions of Major impacts was determined to be related to at least two phenotypic traits. Of this QTL, QTL05 had the most related features (seven). In the study, leaf budding time and flowering characteristics were determined in *Juglans regia* L. using the GWAS method, and it has a powerfull possible to be practical effectively in walnut breeding studies (Bükücü et al. 2020).

4.8. Walnut Genome Sequencing

With the development of high-throughput sequencing tools, rapid progress has been made in the area of chloroplast genetics and genomics. Today, the chloroplast genomes of approximately 800 plant genomes have sequenced and their information has been uploaded to the NCBI gene bank (Daniell et al. 2016). The knowledge obtained from the chloroplast genome has advanced his perspective on all issues related to the plant genome. Besides all this, the sequencing of the chloroplast genome has enabled the identification of not only sequences but also structural differences across plants and plant species (Daniell et al. 2016; Hu et al. 2016, 2017b). Sequences of the chloroplast genome contain important information to figure out the adaptation of plants to adverse environmental conditions and to assist in the reproduction of close related plant species (Daniell et al. 2016). The first chloroplast genome sequencing study in walnut was done by Liu et al (2012) carried out by. Illumina MiSeq tools was used for sequencing and assembled using SPAdes and CpGAVAS programm, respectively. As a result of sequencing, the long of the chloroplast was found to be 160,367 bp, while the GC content was determined as 36.11%. In addition, they determined that 12 protein-coding genes, 14 tRNA and 8 rRNA genes were transcribed in the inverted repeat (IR) regions from the study. In a later study, Hu et al. (2016) Phylogenetic study of the walnut chloroplast genome by 11 chloroplast genomes off different species disclosed that the walnut is close assotiations with the Fagaceae family and the Populus genus in by comparison other species.

In recent years, chloroplast genomes of 5 species of juglans have been sequenced to discover structural models of all chloroplast genomes to define SSRs and to explore divergence hotspots and kinship relationships. Phylogenetic analysis results strongly assistanced that 5 walnut species were divided into Juglans/Dioscaryon and Cardiocaryon 2 divisions as previously determined. (Hu et al. 2017a, b). In another study, wholl chloroplast genomes and 2 core DNA areas of 10 symbolize taxonomic group of Juglans were utilize for kinship relationships of the genus Juglans. It disclosed that 10 chloroplast genomes have 112 genes, containing 78 protein-coding genes, 30 tRNA and 4 rRNA genes. Additionally, Juglans is divided into 3 arms according to 2 nuclear DNA areas; Juglans, Cardiocaryon and Rhysocaryon. (Dong et al. 2017).

The first draft genome of *Juglans regia* (Chandler) was performed utilizing the Illumina sequencing tools and 500 million reads were performed at 120x. The core genome of *J. regia* is 667 Mbp in long by N50 scaffold size 464,955 bp, 221,640 contigs and 37% GC ingredient. Nearly 1.2 million SNPs were explored in the outline consensus sequence genome of *J. regia* (Martínez-García et al. 2016). The genome assembly was made utilizing two diverse tools: SOAPdenovo2 (Luo et al. 2012) and MaSuRCA (Zimin et al. 2013). The discovery of the provincial walnut genome sequence (cv.

Chandler v1.0) supported subsequent walnut genome studies. However, this first sequencing study was performed with short-read sequencing.

The ultimate sequencing and optical mapping attempt have utilized to tide over the restrictions of these technologies and develop the property of the initial genome assembly. Initially, a another genome sequence of Chandler was get utilized in the Oxford Nanopore MinION sequencing tools, outcoming in approximately 7 million reads and 35X genome comprise. The enhanced high-quality walnut genome (cv. Chandler v2.0) will supply a precious genomic means for walnut genetics and genome examinations (Neale et al. 2017). The genomes of plants are generally formed by crossing two different haplotypes in a heterozygous diploid. To conduct research on this, Zhu et al. (2019) conducted a genome study by obtaining an occurring between different species crosses of Juglans microcarpa and *J. regia* cv Serr. Bu çalışmada, bu stratejinin, farklı türler arasında melezlemelerin olduğu veya oluşturulabileceği herhangi bir heterozigot ağaçta etkin bir şekilde uygulanabileceği gösterilmiştir.

Using the previously published walnut reference genome (Chandler v2.0), Oxford Nanopore long-read sequencing was are assembled with chromosome conformation capture (Hi-C) methods and assembled on a new chromosome basis. Compared to the old genome, the new splicing was found to have an 84-fold rising in the read at N50, with 16 chromosomal pseudomolecules and representing 95% of the total length. Single-molecule real-time sequencing utilizing whole transcripts efficiency 37,554 gene patterns with upwards mean gene long than former gene annotations. And then trialed the possible effect of the novel chromosome-level genome on diverse fields of walnut examinations.It was observed that the proteome from Chandler v2.0 available fewer artifacts on male flower growth than the prior to the reference genome, making possible the recognition of a novel possible pollen allergen. Generally, Chandler v2.0 will make the service a worthy source to preferable figure out walnut genetics (Marrano et al. 2020).

Complete structural and functional genome annotations of 6 Juglans species and 1 group from the Juglandaceae family, generated utilizing the BRAKER2 semi-supervised gene platform, were studied. For every annotation, gene give notice were sequence employ19 tissue-private *J. regia* transcriptomes arranged to the genomes. Researchers used this high-quality data to appraise the evolution of genes inside Juglans and between Juglans and Eurosid species. Researchers studied to determine remarkable conjunctions in some gene families in *J. hindsii*, containing disease resistance-associated with wall-associated kinase, Catharanthus roseus receptor-like kinase and another included in abiotic stress reply. Eventually, he approved an old all-genome copy that get placed in a joint ancestor of Juglandaceae utilization area replacement compare analysis (Trouern-Trend et al. 2020).

4.9. Walnut Genome Resequencing

To determine allelic variants in *J. regia* genetic variation was performed using sequencing and resequencing methods. In a genome resequencing study menagemented at UC Davis, they aimed to explore whole genome sequence changes in 27 participants in walnut breeding programs. These genomic knowledge will be an significant source for forward genetic and population examination to determine alleles related with phenotypes in the walnut germplasm (Marrano et al. 2019).

4.9.1. SNP exploration and investigate the genetic constitution with SNP: After resequencing the walnut genome, the BWA-MEN toolkit was utilizing to align the reads to the SNP and reference genome, resulting in 17,800,528 SNPs, of which 609,658 SNPs were employed for the last sequence project process. (Marrano et al. 2019).

4.9.2. SNP Genotyping This new Axiom® Walnut700K, It was applied to a total of 1284 tree investigate the genetic constitution of in the walnut breeding study at UC Davis, as well as 95 walnut tree populations gathered from distinct areas of Iran (Arab et al. 2019; Marrano et al. 2019). The outcomes indicated that a great part of SNPs, not only for UC Davis but also for Persian walnut trees, join the group of polyHigh Resolution (PHR) polymorphisms. The results demonstrated that the Axiom® Walnut700K SNP sequence is an effective genomic instrument for GWAS examination and walnut genetics studies (Marrano et al. 2019; Neale et al. 2017).

4.10. Genomics-Assisted Breeding in Walnut

The development of next-generation sequencing technics has enabled the exploration of SNPs in fruits and vegetables, eased genome-wide association studies (GWAS), and make possible marker-assisted selection (MAS) and genomic selection (GS) Genome-wide association studies (GWAS) happened crucial for the finding of aplicant areas related to easy and complicated characters. Genomic choosing moves forward and chooses genotypes that are forecasted to be excellent rated on their genomic prediction reproductive values. (Iwata et al. 2016; Laurens et al. 2018; Ru et al. 2015; van Nocker and Gardiner 2014). They hinder the traditional genetic development of fruit and nut trees due to their long juvenile sterility and heterozygotic nature. Additionally, trees are also influenced by biotic and abiotic conditions that make genetic development difficult (Rikkerink et al. 2007). By speeding up the reproductive period, shortening the juvenile infertility cycle, and providing early selection with methods such as GWAS and GS, genetic development can be accelerated. Therefore, linked to the genomic paths are of great importance in increasing the yield of genetic progress in fruit and nut trees (Ru et al. 2015).

4.11. Functional Genomics: Transcriptomics

Various genomic tools are utilized to investigate the transcriptome in the course of the progress of plants or as a rely of harsh environmental conditions. cDNA-amplified fragment length polymorphism (AFLP) study by Bâaziz et al (2012) examined walnut leaves held in the light and in the dark to define first molecular incidents in the course of light-induced leaf hydraulic conductivity (Kleaf). The results of the examination indicated that some of the transcript-derived fragments (TDFs) acquired through cDNA-AFLP coincide to 57.9% of protein yields and genes included in general metabolism, respectively.

The early structure of walnut ESTs in *Juglans regia* off seed coat matters by Muir et al (2004) was delivered to the NCBI data bank (https://www.ncbi.nlm.nih. gov). Other study, functional genomic analysis to define the genes included in coactions across the walnut root and the nematode was made at UC Davis. In this study, in firstly, 13,559 ESTs were created with data cDNA libraries supplied from Pratylenchus vulnus and infected and uninfected walnut (*J. hindsii* × *J. regia*) leaves and roots (Britton et al. 2007). Secondly, it is made to define and approve nematode and walnut genes related to the infection study of gene expression across uninfected and infected plants. Consequently, functional analysis of nematode genes was made utilizing RNA interference in vitro and Medicago root analysis (Britton et al. 2009). Besides in this study, 2733 P. vulnus genes and 8622 walnut genes were sequenced and exhibited valued knowledge on the ways included in the interplay across P. *vulnus* and a sensitive walnut rootstock

Zhang et al. (2010) acquired a sum of 5025 ESTs for walnut off the NCBI databank and utilizing to examine SSR motifs by the SSR Hunter program. Afterward, a sum of 123 primer pairs was developed of the non-redundant SSR-including unigenes. The efficacy of candidate markers was researched by composing seven DNA pools from walnut genotypes attained from varied regions. The results disclosed that 41 SSR primer pairs with high polymorphism of the amplified yields may be utilized for coming genetic investigating in walnut.

In other study, 5213 EST data of walnut (*Juglans regia*) in NCBI were employed for improving walnut EST-SSRs, and 207 SSRs were getting from the EST sets. Total 7262 unigenes were getting from 13,559 ESTs received off the NCBI data bank and 309 EST-SSR primers were casually arranged. Consequently, 13 very polymorphic EST-SSR marker was employed for genetic investigation in *Juglans regia*, *J. nigra*, *Carya cathayensis, C. dabieshanensis* and be in danger species *Annamocarya sinensis* (Zhang et al. 2013). Furthermore, 40 polymorphic EST-SSRs were improve for J.regia by Zhao et al. (2015). Latterly a few transcriptomes investigate have performed in walnut which comprises expression of the transcription factor gene JrCBF contained in cold reluctance contraption (Xu et al. 2014). Additionally, expression of a great family of NBSLRR reluctance genes in *J. regia* comprised in plant-microbe co-action (Chakraborty et al. 2016) and transcriptome examine of buds, leaves, female flowers and male flowers in *J. regia* was utilized to define novel EST-SSRs (Dang et al. 2016). Furthermore, Li et al. (2018) performed a comparison transcriptome examination of genes inclusive in anthocyanin biosynthesis in leaf and peel color change in red and green *J. regia.*

Phenylalanine ammonia lyase (PAL) is initial enzyme in the phenylpropanoid pathway, critical in plant progress, conformation and improving, however, few studies have recorded on the PAL gene family in walnuts. In this study, researchers record genome-wide investigation of *J. regia* PAL genes and examine their phylogeny, replication, microRNA and transcriptional expression. Totally 12 PAL genes were determined in the widespread walnut and group into two subfamilies relyed on phylogenetic investigation. This widespread walnut PALs are deployed on eight distinct pseudo-chromosomes. Seven of the 12 PALs were exclusive determined in *J. regia*, and five PALs gene were defined to be nearly related to the woody plant Populus trichocarpa. In additional to the expression types of four PALs gene indicated that they had up expression in female and male flowers. The miRNA ath-miR830-5p regulates two genes, such that they have down expression in the male and female flowers of the widespreat walnut. Results of research, supplies beneficial knowledge for upwards expression in the task of PAL genes in widespread walnut and Juglans (Yan et al. 2019).

In examination, well-analysed the evolutionary advancement of the plants of the Juglandaceae are established related on both the core RAD-Seq and the entire chloroplast genome seq. Results assistance the Juglandoideae study of geometric properties (Hicoreae, (Platycaryeae, Juglandeae)) at the popülation level. Inside of Juglandeae, a incompatible location of Pterocarya was determined across nuclear and plastid genome information, and a more probably topology (nuclear), was debated relayed on proof off molecular information and fossil reports. Relied on cautiously chosen fossil adjustments, the remove times of surviving progeny were predicted and they were confirmed well on fossil records (particularly regarding Juglans and Pterocarya). Four departments into Juglans were vigorously assistanced by the core information. Into Juglans, the incompatible location of J. hopeiensis was obtained across the nuclear and plastid genomes. in this study indicates the potency of planning to unify separate biogeographic examination for illuminating the evolutionary date of Juglandaceae (Mu et al. 2020).

In this study produced 240,179,782 reads from 11 walnut leaves by cDNA data. The reads supply a whole de novo transcriptome installing. Fifteen diverse transcriptome assemblies were established from 5 distinct reputed assemblers employ in academic literature by distinct k-mer longs (Bridger, BinPacker, SOAPdenovo-Trans, Trinity and SPAdes), additionally two combining perspectives (EvidentialGene and Transfuse). Relied on the 4 feature measures of assembly, the outcomes showed an yield in the continuum of combining the assemblies then being created with de novo assemblers. Eventually Evidential- Gene was realized as of the first assembler for the de novo installation of the leaf transcriptome in walnut. Between totally number of 183,191 transcripts which were created with EvidentialGene, there were 109,413 transcripts talent of protein potency (59.72%) and 104,926 were realized as ORFs (57.27%). Also, 6,591 of the foretelled peptide datas included marker peptides, as 92,704 included across a cell membrane areas. Compared the consubstantiate transcripts with transcripts of the walnut and published genome installation for the ‘Chandler’ plant variety utilizing the BLAST algorithm to cause determine a totally of 27,304 and 19,178 homologue transcripts, respectively. De novo transcriptomes in walnut leaves may be improved for the forthcoming examination in convenient genomics and genetic investigation of walnuts (Sadat-Hossein et al. 2020).

İn order to figure out the molecular treatment fundamental the advocating of walnut to C. gloeosporioides, they have been utilizing RNA-seq. Totally, 21,798 in a varied way stated genes (DEGs) and 1929 a varied way stated proteins (DEPs) were determined in F26 vs. F423 at five-time periods, and DEGs and DEPs expressions were effectively upward of in the first infection phase. Determined 2 modules' important associations with disease reluctance and 9 hub genes in the transcription statement gene systems. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis of the DEGs and DEPs disclosed that very genes were most connected to immune reply, plant hormone signaling transduction, and second metabolites, and many DEPs were contained in carbon metabolism and photosynthesis. As a result, assistances brighten the molecular reply of walnut fruit to C. gloeosporioides and supply a foundation for the genetic progress of walnut disease reluctance (Fang et al. 2021).

5. RESULTS

Walnut is one of the hard-shelled fruits that have been very popular in recent years due to its high nutritional content, important economic value, and valuable timber. The significant improvement in walnut growing and the promotion of property and efficient diversity has caused a rising up in its breeding. High output and features have every time been fundamental walnut growing targets. High yield on lateral branches, excess nut weight, large fruit, the high kernel rate, delay leafing, early harvest, and light kernel color are the main quality characteristics targeted in walnut. Manufacturers have utilized different methods to succeed in these targets, like germplasm evaluation and choosing, cross, genetic engineering, variation breeding, genome sequencing, marker-assisted selection, haploid, and polyploid causations, proteomics, and metabolomics. Now walnut growing methods center on combining molecular breeding (utilizing genomic, transcriptomic, proteomic and metabolomics knowledge) into conventional breeding methods. Climate condition change and global heating may seriously affect global walnut generations. For this reason, walnut growing methods, particularly in the important and best active territories, are struggling to create walnut varieties rapidly and effectively that protect and continuously develop walnut yield and property. According to this, utilizing of CRISPR-Cas9 methods for goal-genome regulation, have generations of different chromosome number plants to increase genome installation feature, joining high-throughput the set of observable characteristics of an individual with GWAS and genomic choosing, utilizing of transcriptomics, metabolomics and proteomics for comprehension to hard environmental conditions resistance operation in walnut, continuous assessment of genetic variation to obtain and improve novel walnut variety and rootstocks and the introduction of varieties that are resistant to adverse environmental conditions and of high quality value are priorities for future walnut breeding programmes.

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Part XIII

Secondary Metabolites in Walnut

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Introduction

The importance of walnut in the human diet

Secondary metabolites, especially phenolic compounds, are of particular importance in walnut trees because of their significant role in physiological processes. The nutritional content of walnut has made it the most preferred food in a healthy diet. The walnut fruit contains a high rate of fat and protein. On the other hand, almost all parts of the tree have been used in folk medicine since the ancient era.

Walnut (*Juglans regia* L) fruit is among the strategic products in human nutrition due to being a rich source of phytochemical

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compounds. Walnut (*Juglans regia* L.) is a member of the relatively small family Juglandaceae, a set of economically very important tree species (Guney et al., 2021a, b). The importance of walnut consumption becomes more prominent in the prevention of cardiovascular diseases, lowering cholesterol and triglyceride levels, a rich source of silver and selenium which are important for child nutrition (Ros, 2010); (Tindall et al., 2019); (Nguyen and Vu, 2021).

Walnut fruits are a type of hard-shelled fruit that has been consumed for centuries due to their rich oil content ranging from 52-70%. Walnut kernel contains a high rate of monounsaturated fatty acid content such as oleic acid, which is of great importance for human health and nutrition, and essential polyunsaturated fatty acids such as linoleic and linolenic acid. The high content of these essential causes fatty walnut fruits to have weak oxidative stability, however, the ability of walnuts to prevent heart disease and reduce blood cholesterol levels is due to their oleic acid, linoleic acid, and linolenic acid content (Arcan et al., 2021); (Martinez et al., 2006). The derivatives of vitamin E, Tocopherols and tocotrienols, act as antioxidants and have a wide range of physiological, biological, and biochemical functions (Bozkurt and Ergun, 2021); (Okatan et al., 2021) (Kafkas et al., 2020). Furthermore, the most important biochemical function of tocopherols is to prevent the peroxidation of polyunsaturated fatty acids. Alpha-tocopherol is known as the most necessary form of vitamin E for the human body and its deficiency is very common in modern nutrition. Vitamin E, known as antioxidant polyphenol, has been reported to prevent heart and cancer diseases (Kodad et al., 2014).

Secondary metabolites and their classifications

Constant and inevitable changes in the ecosystem require changes in biological and physiological processes to increase the adaptation of plants to these changes and to cope with the existing stresses.

The plants apply various mechanisms of adaptation to the abiotic, biotic, and anthropogenic factors by synthesizing numerous organic compounds. The organic compounds Synthesized in plants are divided into three main groups. The first group, primary metabolites such as carbohydrates, proteins, and fats, is directly involved in normal plant growth, development, and reproduction. The second one, phytohormones, is chemical messengers responsible for coordinating cellular activities and affect physiological processes occurring naturally at very low concentrations in plants. phytohormones are (Gundesli et al., 2020a,b,c); (Su et al., 2017). Contrary to primary metabolites, secondary metabolites as the other group of plant phytochemicals, do not participate directly in the developmental or reproductive stages of a plant and the absence of secondary metabolites does not lead to immediate cell death but may influence the survival of the plant and its fertility in a long time. These metabolites are countable for various physiological and ecological functions such as regulating of plant growth and developmental processes such as attracting insects for pollination by color and volatiles, defense system against pathogens antifeedants and allelochemicals against herbivores, innate immunity, response to environmental stresses such as temperature, water deficiency, ultraviolet radiation, elicitors, and acting as signals for symbiosis between plants and microorganisms (Piasecka et al., 2015); (Yang et al., 2018); (Guerrieri et al., 2019); (Isah, 2019); (Bodoira and Maestri, 2020); (Corso et al., 2020); (Yuan and Grotewold, 2020); (Revutska et al., 2021); (Pang et al., 2021). Secondary metabolites also are applied in the pharmaceutical and food industries and play significant roles in the human health diet (Ergun and Zarifikhosroshahi, 2020); (Ullrich et al., 2019); (Fakhri et al., 2020).

Secondary metabolites are derived from the biosynthesis pathways of primary metabolites and are considered as their byproducts. Although primary metabolites are synthesized in the cells of all plant species, secondary metabolites may be characterized by specific species. However, despite a huge variety of known secondary metabolites in plants, the exact functions of many are still unknown (Kosmacz et al., 2020). Secondary metabolites are mainly divided into three classes of terpenoids (≈25,000 compounds), alkaloids (≈12,000 compounds), and phenolics (≈8,000 compounds) (Croteau 2000). However, xanthones, quinones, glycosides, steroids, lignans, coumarins, etc are among known plant secondary metabolites. Terpenoids are the most structurally diverse natural plant compounds. These compounds are commercially important due to their wide range of applications in industrial products, including seasonings, drugs, perfumes, insecticides, and antimicrobials. Terpenoids are used in industry for their unique properties as biological materials for the production of heavy tires, shock absorbers, and latex products such as surgical gloves (Kim et al., 2011).

Alkaloids are compounds that have at least one nitrogen atom in a heterocyclic ring. These secondary metabolites play a predominantly defensive role and are sometimes used as sources for nitrogen storage in plants. These compounds have a bitter taste and have a toxic effect on the cell membrane system, especially nerve cells. They act as an insecticide and are very important in the pharmaceutical industry. Extensive studies have shown the diverse biological activity of indole alkaloids, from antibacterial activity to anti-inflammatory activity and anti-tumor activity. In addition, indole alkaloids from the marine environment are a promising and active group of biomolecules that cover biological, cytotoxic, antiviral, antiparasitic, and anti-inflammatory activity (Tadeusz, 2007).

Flavonoids cause color in flowers, fruits, and sometimes leaves. They are also effective in the pollination and fertility of plants due to their ability to attract insects. Flavonoids increase resistance to plant pathogens and are also strong absorbers of ultraviolet light (340-250 nm). In the structure of sexual and vegetative organs, pollen grains have a positive effect on the action of genes and enzymes. It also chelates some metal ions such as iron and copper. Flavonoids prevent oxidation by inhibiting catalyzed elements. Other properties of these materials include waste collection properties. Flavonoids are inhibitors of cancer cells that exert this effect through gene expression, boosting the immune system (antioxidant), antiviral, antibacterial, anti-inflammatory, anti-inflammatory, anti-allergic, anti-mutation, permeability, and capillary fragility (Nijveldt et al., 2001).

Methods for identification of secondary metabolites

Considering the value of secondary metabolites, a variety of laboratory methods have been introduced to study the structure of these compounds. Many of these methods are costly and time-consuming if done for the first time. Therefore, one of the advantages of identifying the structure of secondary metabolites is the possibility of creating virtual libraries of information from these compounds. Hence, compounds can be studied with more cost-effective devices.

Identification of the chemical structure also provides an opportunity to study the ligand-enzyme properties *in vitro*. The most widely used methods include gas chromatography-mass spectrometry (GC/MS), liquid chromatography-MS (LC-MS), capillary electrophoresis-MS (CE-MS), nuclear magnetic resonance spectroscopy (NMR), Fourier transform-near-infrared (FT-NIR) spectroscopy, MS imaging (MSI), and live single-cell-MS (LSC-MS) (Pang et al., 2021); (Zarifikhosroshahi and Ergun, 2021).

In mass spectrometry, one or more atomic ions are separated based on the mass-to-charge ratio (m/z) and measuring m/z and the frequency of ions in the gas phase. More precisely, mass spectrometry examines the mass-to-charge ratio of molecules using electric and magnetic fields. This method is one of the most widely used methods in identifying metabolites. The main advantage of this technique is its high sensitivity. In addition, combining the MS method with chromatographic separation increases the accuracy and high ability to separate and identify compounds in the existing metabolite (Krug and Müller, 2014). The two combined methods GC-MS and LC-MS are the most widely used. The GC-MS consists of a combination of gas chromatography (to separate gas mixture components) and mass spectrometer (to identify components) and is a widely used method for the separation and identification of volatiles. The LC-MS method is also one of the most widely used methods in studies related to metabolites in plants. The main method for ionization in this technique is API, which includes two methods EI and APCI (Dunn, 2008).

Nuclear magnetic resonance spectroscopy is another popular method in metabolic studies. This method determines the atomic status of compounds, and is also able to detect metabolites that are not detectable by methods such as MS. NMR spectroscopy can provide accurate information on the amounts and identities of metabolites present in the extract as well as in vivo conditions (Sekiyama et al., 2010). High-pressure liquid chromatography equipped with a UV detector is also a prevalent method for the identification of phenolic compounds and flavonoids (Ergun, 2021 a,b).

Detected secondary metabolites in different parts of the walnut tree

Based on the previous studies, bioactive compounds such as polyphenols and sterols contribute to the health-promoting properties of walnuts (Ni et al., 2021).

Phenolic compounds

Phenolics are organic compounds formed by the bonding of hydroxyl groups and aromatic rings. Phenolic compounds have been classified into different sub-classes such as phenolic acids, flavonoids, stilbenes, coumarins, and tannins. Walnuts are a rich source of phenolic acids, flavonoids, ellagitannins, and gallotannins. Phenolic compounds are involved in the regulation of physiological pathways in the growth and development of plants. Health-promoting effects of walnut extremely are due to polyphenol content.

The main component of phenolics is trihydroxy benzoic acid (gallic acid) (Nieto et al., 2020). By hydrolyzation of gallic acid, ellagitannins and gallotannins are produced. Ellagitannins are more stable than gallotannins due to covalent and hydrogen bonds in ellagitannins within carbonyl and hydroxyl groups. Ellagitannins such as casuarictin, gitannins, pedunculagin and tellimagrandins are the main group of phenolics in walnut kernels (Anderson et al., 2001);

(Fukuda et al., 2003); (Cerdá et al., 2005). Ellagitannins are esters of hexahydroxydiphenic acid and a polyol such as glucose and can be hydrolyzed by acids or bases to break down into ellagic acid (Gómez-Caravaca et al., 2008); (García-Villalba et al., 2015). Besides ellagic acid which is found both in the form of Ellagitannins and free form, gallic acid, syringic acid, vanillic acid, p-coumaric acid, and ferulic acid, were also found abundantly in walnuts. Although glycosylated forms of phenolic acids are also found in walnut, they are not desirable compared to free forms because the researches proved that the addition of glucoside decreases the biological and pharmacological activities of phenolic acids (Kumar et al., 2013).

Catechins, procyanidins, epicatechin gallate, quercetin, quercetin- 3-β-D-glucoside, and rutin are also flavonoids that have been detected in different walnut species. Walnut kernels are also rich in tannins. Tannins are divided into two classes, condensed tannins (proanthocyanidins) and hydrolyzable tannins (Ossipov et al., 2003). Condensed tannins are polymeric flavonoids composed of at least two linked catechin units (Khanbabaee and Ree, 2001). Despite the low percentage of the pellicle (Figure 1) compared to the total mass, walnut pellicle contains more tannin in walnut (Alasalvar and Shahidi, 2010). Walnut green husk contains a large amount of quinines (Maleita et al., 2017); (Meshkini and Tahmasbi, 2017). Most quinones have an unsaturated cyclic dione structure in the molecule, and anthraquinone and its derivatives are particularly important in traditional Chinese medicine (Beiki et al., 2018). The main quantity of phenolic compounds of walnuts is mostly presented in the hull (Fukuda and Yoshida, 2003). Pellicles commonly have a higher amount of phenolics compared to the kernel without pellicles. The flavanols levels were 77 times more in the pellicle compared to kernel without pellicle (Shen et al., 2021).

Thirty-four phenolic acids were detected by Shen et al., (2021) in kernel and pellicles of walnut. The walnut pellicles contained high levels of ellagic acid, catechin, (-)-epicatechin gallate, and catechin gallate. However, ellagic acid was identified as the main phenolic compound in kernels without pellicles. Shen et al., (2021) also detected ferulic acid, chlorogenic acid, caffeic acid, and protocatechuic acid, kaempferol, luteolin, and vitexin in the walnut kernel. Hydrolyzable tannins, phenolic acids, and flavonoids are richly concentrated in walnut pellicles (Alasalvar and Shahidi, 2010). Zhang et al., (2020) detected about 32 phenolic compounds in walnut pellicle of which ellagic acid was the major one.

Ni et al., (2021) reported that walnut kernels contain phenolic acids including hydroxybenzoic acid, cinnamic acid, coumaric acid, syringic acid, derivatives and three unknown compounds. These derivatives comprise syringic acid, gentisic acid, gallic acid, 3-coumaric, 4-coumaric, and dihydro-p-coumaric acid. Vu et al., (2018) also detected 16 phenolics in 11 different black walnut cultivars including phenolic acids, flavonoids, and catechins. Ellagic acid was the predominant phenolic compound in all cultivars. Despite differences among cultivars, p-Coumaric acid, quinic acid, Gallic acid, and (−)-

Epicatechin gallate were detected in the majority of cultivars. Significant differences were identified in the quantities of gallic acid, quinic acid, 1,3,6-trigalloylglucose, catechin, and Penta-O-galloyl-βD-glucose between the studied black walnuts and English walnut.

Regueiro et al., (2014) detected a wide range of phenolic compounds (120 compounds) including hydrolyzable and condensed tannins, flavonoids, and phenolic acids of some which have never been detected before (stenophyllanin C, malabathrin A, eucalbanin A, cornusiin B, heterophylliin E, pterocarinin B, reginin A and alienanin B) in walnut kernel by "electrospray ionization hybrid linear trap quadrupole-Orbitrap mass spectrometry" (LC–LTQ-Orbitrap). Among detected phenolics (Chlorogenic acid, Neochlorogenic acid, 3-O-p-Coumaroylquinic acid, 4-O-p-Coumaroylquinic acid, Coumaric acid hexoside, Gallic acid, Ethyl gallate from phenolic acids; Catechin, Epicatechin gallate, Procyanidin dimer, Quercetin, Quercetin galloylhexoside, Quercetin hexoside, Quercetin pentoside, Monogalloylglucose, Digalloylglucose, Trigalloylglucose, Tetragalloylglucose, Ellagic acid, Ellagic acid pentoside, Ellagic acid hexoside, Glansreginin, Casuariin, Tellimagrandin II, Heterophylliin D, Pedunculagin, Casuariin, Strictinin, Rugosin, Casuarinin, Stenophyllanin, Pterocarinin, Platycaryanin A, Euprostin A, Flavogallonic acid dilactone, Alienanin. Oenothein, Reginin, Malabathrin from Flavonoids), ellagitannins, ellagic acid, and its derivatives were the most abundant.

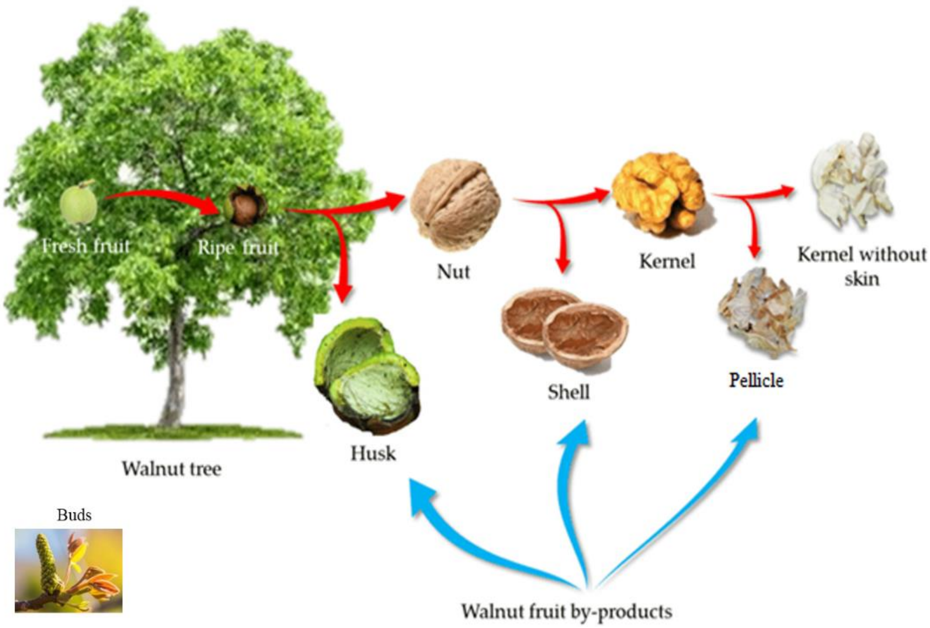

Figure 1. Different parts of walnut fruits.

For the first time, Medic et al., (2021) identified the phenolic profile of bark, husks and buds of walnut in six cultivars and detected 29, 38, and 57 phenolics compounds, respectively. Of 83 total phenolics, 25 naphthoquinones, 15 hydroxycinnamic acids, 8 hydroxybenzoic acids, 13 flavonols, 2 flavones, 1 flavanone, and 19 flavonols were detected. In all three parts, naphthoquinones were the main phenolic compound. Juglone is a naphthoquinone that is characterized as the main phenolic in the species of Juglandaceae family (Figure 2). Naphthoquinones take part in various oxidative processes, act as links in the transportation of electrons, and might also play role in defensive mechanisms in interspecies chemical competition such as allelopathy. Naphthoquinones also are important in pharmaceutical industries in developing natural base drugs as antibacterial, antifungal, antiviral, antiparasitic, and antitumor (Pinho et al., 2012).

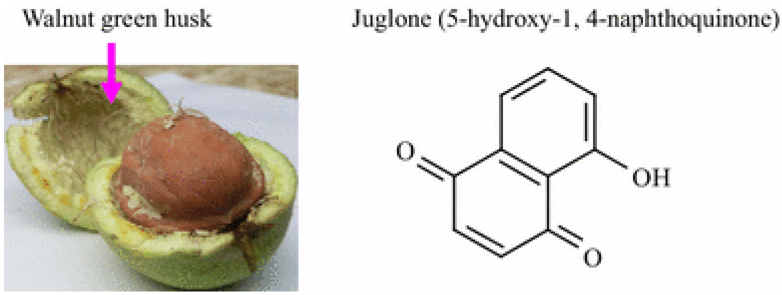

Figure 2. The sturucture of Juglone

Soto-Madrid et al., (2021) also detected 9 polyphenols including Gallic acid, Protocatechuic acid, Catechin, Caffeic acid, Ferulic acid, Polydatin, Hesperetin, Resveratrol, Quercetin, Myricetin, Kaempferol, Hesperidin, Juglone in the husk of walnut. Previously, Shi et al., (2017) also have been reported that among different phenolic compounds detected in walnut husk, (gallic acid, chlorogenic acid, catechin, caffeic acid, vanillic acid, syringic acid, epicatechin, ferulic acid, syringaldehyde, rutin, ellagic acid, quercetin, myricetin, and juglone) juglone was the predominant phenolics.

Gutiérrez et al., (2018) identified hydroxycinnamic acid, chlorogenic acid, caffeic, ferulic, p-coumaric and sinapic acids, cis and trans-mono-caffeoylquinic, dicaffeoylquinic, mono-feruloylquinic and cis and trans-mono-p-coumaroylquinic acid isomers by Ultra High-Pressure Liquid Chromatography.

Phenolic data in the aforementioned studies have demonstrated that phenolic contents differ among walnut cultivars. In other words, the variations in phenolic compositions are ascribable to genetic traits. There is increasing evidence to suggest that levels of phenolics in walnuts may also be affected by agronomic practices, environmental and climatic conditions and geographic factors (Nguyen and Vu, 2021); (Toide and Tajima, 2015); (Fuentealba et al., 2017); (Cohen et al., 1996); (Lynch et al., 2016).

Phytosterols

Phytosterols are a type of lipid molecule that possess structural and functional similarities to cholesterol. phytosterols are common in nut kernels. Weihrauch and Gardner (1978) reported the presence of three phytosterols (β–sitosterol, campesterol, and stigmasterol) in the kernel of walnut for the first time. Later, various types of phytosterols were identified in walnut. Thirteen phytosterols were detected in walnut kernels by Abdallah et al., (2015) in Tunisia. Among reported phytosterols, β–sitosterol was the major one with 63 – 89% of the total sterol levels quantified in walnuts (Phillips et al., 2005); (Robbins et al., 2011); (Vu et al., 2019); (Abdallah et al., 2015).

Studies proved that Phytosterols as well as β–sitosterol has the ability to lower cholesterol level in the human body. Beta-sitosterol has also been shown to improve Benign Prostatic Hyperplasia (Pizzorno and Murray, 2020). Significant variations in individual and total phytosterol levels of walnut were reported in previous studies. These differences were attributed to genetic factors, geographic origin, environmental factors, and seasonal variations (Gong et al., 2017); (Gong et al., 2017); (Crews et al., 2005); (Rabadán et al., 2018); (Rabadán et al., 2019). The differences in extraction methods, harvest time, and horticultural characteristics are among other factors affecting phytosterols types and quantities in walnut (Nguyen and Vu, 2021).

Tocopherols

Tocopherols are another group of secondary metabolites that occur abundantly in the walnut kernel. Tocopherols are liposoluble, natural compounds with high antioxidant activities which comprise α, β, γ, and δ tocopherols. Tocopherols donate the hydrogen of the hydroxyl group to the lipid peroxyl radical. Among different tocopherols, α-tocopherol is the most important one due to vitamin E activity (Beyhan et al., 2016).

Studies showed four different isomers of tocopherols (α-, β-, γ- and δ) detected in walnut oils. Among detected tocopherols in walnut kernel oil, γ-Tocopherol is the predominant form (Robbins et al., 2011); (Beyhan et al., 2016); (Arcan et al., 2021). However notable variations are reported in tocopherol contents of walnut kernels by researchers. Tocopherol levels were extremely affected by cultivars, genetic and environmental factors, and harvest time (Özrenk et al., 2012); (Beyhan et al., 2016).

Tocotrienols, lipid-soluble compounds, are also other secondary metabolites detected in walnuts (Li et al., 2006); (Matthäus et al., 2018); (Robbins et al., 2011); (Amaral et al., 2005a, b). Tocotrienols are also from the vitamin E family with antioxidant capacity. The difference between tocotrienols and tocopherols is related to their chemical structure in which tocotrienols have unsaturated isoprenoid side chains with three carbon-carbon double bonds versus saturated side chains for tocopherols (Kamal-Eldin and Appelqvist, 1996). However, limited information about the presence of these compounds in walnuts is available.

Plant Hormones

Phytohormones also as a group of secondary metabolites are involved in the regulation of the metabolic activities within cells and the development of the plant. The regulators of plant growth and development play a key role in managing the stress signals and biochemical and physiological pathways (Hasanuzzaman et al., 2020). Melatonin is not only a phytohormone, it is released by the pineal gland in the human body at night and controls the sleep-wake cycle (Auld et al., 2017). Melatonin is also involved in various functions in improving the performance of the mitochondrial electron transport chain and antioxidant activity (Ahmad et al., 2020); (Jaferi and shahsavar, 2021). Reiter et al., (2005) reported that walnuts are a good source of melatonin and when eaten the concentrations of blood melatonin increased which was associated with the increased antioxidative capacity of the blood. Later, several studies identified the amount of melatonin in walnut (Reiter et al. 2005); (Tapia et al., 2013); (Pycia et al., 2019); (Paroni et al., 2019); (Wang et al., 2020); (Verede et al., 2021). It is reported that the content of melatonin differs extremely in some nuts studied in different researches (Reiter et al., 2005); (Tapia et al., 2013); (Kocadağlı et al., 2014); (Paroni et al., 2019). These differences may due to factors such as the cultivar, variety, harvesting time, and the fruit maturity (Kocadağlı et al., 2014) besides the status of nuts consumption (Chang et al., 2016); (Paroni et al., 2019) and extraction methods (Verede et al, 2021).

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Part XIV

Fatty Acids Composition of Walnuts

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1. Introduction

Lipids are compounds found in plant and animal cells that are insoluble in water but soluble in nonpolar solvents such as chloroform, ether, and hexane (Korkmaz et al., 2012). They form our body's largest source of energy. All animal and plant foods contain different amounts of oil. One of the food groups that should be consumed daily to provide adequate and balanced nutrition is oilseeds. Oilseeds have considerably rich content in terms of B vitamins, oil, protein, and minerals. Vegetable products such as walnuts, pistachios, peanuts, almonds, and pumpkin seeds are taken part in the group of oil seeds (Ayaz, 2008; Gundesli et al., 2021).

Walnuts are prevalently consumed due to their substantial nutritional components (Esen, 2013). As well as consuming directly as nuts, it is an indispensable component of many products in the food sector, too. It is used in the manufacture of foods such as many desserts, cakes,

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and cookies, especially baklava and kadayif. Walnut is also an essential material for the pharmaceutical, cosmetic, and chocolate industries (Yan et al., 2021; Demirağ, 2019; Guney,et al., 2021). In addition to many components, fatty acids are also effective in the demand for walnuts as a nutritional source. The high level of polyunsaturated fatty acids in walnut oil has made it a valuable and preferred food. The major fatty acids found in walnut oil are oleic, linoleic and linolenic acids. These polyunsaturated fatty acids are more desirable because of their health benefits (Uzun et al., 2021; Cunnane et al., 1993; Rabrenovic et al., 2011; Demirag, 2019; Kafkas et al., 2020).

Studies have revealed that walnut consumption reduces plasma cholesterol levels. This is thought to be due to the presence of linoleic and linolenic acids, which are essential fatty acids in the diet, in suitable proportions in walnut oil (Yildiz et al., 2021; Sabate et al., 1993; Abbey et al., 1994; Rabrenovic et al., 2011). Linolenic and linoleic acid, which are essential fatty acids that are not produced by the body but must be taken through food, is also known as omega-3 and omega-6 fatty acids (Harris et al., 2007).

The positive effects of omega-3 fatty acids on health were noticed firstly in studies on the Inuit people of Greenland. Despite their traditional diet with high-fat, Inuits have been observed to be resistant to heart, rheumatism, asthma, and many diseases common in industrial countries. It has been suggested that the reason for this is the widespread consumption of fish meat containing unsaturated fatty acids and the oils of marine mammals (Dyerberg et al., 1975; Anonymous, 2021a). Omega-3 fatty acid consumption is important in the prevention of prostate, breast, lung, and intestinal cancers and both the prevention and treatment of cardiovascular diseases, hypertension, rheumatoid arthritis, osteoporosis, diabetes, asthma, Alzheimer's, depression, and schizophrenia. In addition, it has been reported that it has positive effects on the development of intelligence in the early period, the strengthening of the immune system, nervous system development, and brain functions (Stevens et al., 1995; Simopoulos, 1991; Tatar et al., 2001; Ceylan et al., 1999; Çabuk et al., 1999; Gundesli et al., 2019).

Walnut oil contains higher omega-3 and omega-6 polyunsaturated fatty acids than other nuts (Yildiz et al., 2021, Amaral et al., 2003). In studies conducted on walnuts, the presence of other components besides mono and polyunsaturated fatty acids, which are essential for human health, has been determined, and their impacts on health have been the subject of many research. In this article, studies investigating the fatty acid composition of walnut oil and its effects on health have been compiled.

2. Fatty Acids

Fatty acids are hydrocarbons having long chains of lipid-carboxylic acid found in oils and cell membranes as a component of phospholipids and glycolipids (Anonymous, 2021b). They are represented by the formula R–COOH. The "R" can be a saturated or unsaturated long hydrocarbon chain containing one or more carbon-

carbon double bonds. Fatty acids differ according to chain length and the presence, number, and position of double bonds in the hydrocarbon chain.

The systematic nomenclature of fatty acids recommended by the International Union of Pure and Applied Chemistry (IUPAC) is done by adding the suffix -oic to the end of the hydrocarbon chain name. The IUPAC system names fatty acids just on the basis of the number of carbon atoms, and the number and position of unsaturated fatty acids relative to the carboxyl group. Although the IUPAC nomenclature is technically clear, fatty acid names are long and therefore, for convenience, trivial names are frequently used in scientific articles (Anonymous, 2018). The systematic names, common names, and chemical structure of some common fatty acids are presented in Table 1.

Table 1. Some common fatty acids

Abbreviation	**Systematic Name**	**Trivial Name**	**Chemical structure**
C12:0	Dodecanoic acid	Lauric acid	$CH_3(CH_2)_{10}COOH$
C14:0	Tetradecanoic acid	Myristic acid	$CH_3(CH_2)_{12}COOH$
C16:0	Hexadecanoic acid	Palmitic acid	$CH_3(CH_2)_{14}COOH$
C18:0	Octadecanoic acid	Stearic acid	$CH_3(CH_2)_{16}COOH$
C20:0	Eicosanoic acid	Arachidic acid	$CH_3(CH_2)_{18}COOH$

C24:0	Tetracosanoic acid	Lignoceric acid	$CH_3(CH_2)_{22}COOH$
C16:1	9-*cis*-Hexadecenoic acid	Palmitoleic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
C18:1	*cis*-9-Octadecenoic acid	Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
C18:2	*cis,cis*-9,12-Octadecadienoic acid	Linoleic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
C18:3	*cis,cis,cis*-9,12,15-Octadecatrienoic acid	α-Linolenic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$

Structural variation among fatty acids gives rise to functional differences that result in different impacts upon metabolism, cell and tissue responses (Burdge and Calder, 2015). Structurally, the fatty acids are of two kinds as saturated and unsaturated (Figure 1). Unsaturated fatty acids have mono- and polyunsaturated types, too. Fatty acids that aliphatic carbon chains are fully saturated with hydrogen atoms contain only C-C single, but contain no C=C double bonds are identified as saturated fatty acids. Fatty acids containing C=C double bonds are referred to as unsaturated fatty acids. If they contain only one C=C double bond, they are monounsaturated fatty acids. If they contain more than one C=C double bond, they are then called polyunsaturated fatty acids.

Polyunsaturated fatty acids from nutritional perspectives are categorized as essential and nonessential types. The fatty acids, which mammals cannot synthesize in their body, are known as essential fatty acids; they must be obtained from the exogenous dietary sources. Omega-6 linoleic acid, omega-3 α-linolenic acid omega-6 arachidonic acid are essential fatty acids. Fatty acids that mammals can synthesize in their bodies are called nonessential fatty acids. The polyunsaturated fatty acids that have a C=C double bond between the 6th and 7th carbon position counting from the terminal methyl end are called omega-6 and those with the double bond between the 3th and 4th carbon are called omega-3 polyunsaturated fatty acids (Hashimoto and Hossain, 2018). The classification of the most common fatty acids and their sources are presented in Table 2.

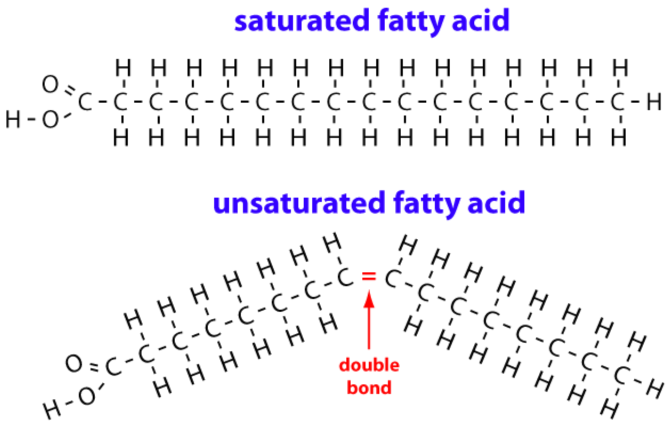

Figure 1. The Structural Configurations of The Saturated and Unsaturated Fatty Acids (Anonymous, 2021c)

Table 2. **Saturated and Unsaturated Fatty Acids and Their Typical Sources** (Doğan and Akgül, 2005; Chisholm et al., 1998; Geng et al., 2021; Kaflas et al.,

2020; Uzun et al., 2021; Ogungbenle and Anisulowo, 2014; Maguire et al., 2004; Nogales-Bueno et al., 2021; Amaral et al., 2003; Savage et al., 1999; Arcan et al., 2021)

Series	**Trivial Name**	**Abbrev**	**Typical sources**
Saturated Fatty Acids	Butyric acid	C4:0	Dairy fat
	Caproic acid	C6:0	Dairy fat
	Caprylic acid	C8:0	Dairy fat, coconut and palm kernel oils
	Lauric acid	C12:0	Coconut and palm kernel oils
	Myristic acid	C:14:	Dairy fat, coconut, palm and walnut kernel oils
	Palmitic acid	C16:0	Most fats and oils (included walnut oil)
	Stearic acid	C18:0	Most fats and oils (included walnut oil)
	Arachidic acid	C20:0	Peanut oil, walnut oil
	Behenic acid	C22:0	Peanut oil, walnut oil
	Lignoceric acid	C24:0	Peanut oil
Monounsaturated Fatty Acids	Palmitoleic acid	C16:1	Marine oils, macadamia oil, most animal and vegetable oils (included walnut oil)
	Oleic acid	C18:1	All fats and oils, especially olive oil, canola oil and high-oleic sunflower and safflower oil, walnut oil
	cis-vaccenic acid	C18:1	Most vegetable oils (included walnut oil)
	Gadoleic acid	C20:1	Marine oils
	Erucic acid	C22:1	Mustard seed oil, high erucic rapeseed oil

	Nervonic acid	C24:1	Marine oils
	α-linolenic acid	18:3ω-3	Flaxseed oil, walnut oil, perilla oil, canola oil, soybean oil
Polyunsaturated Fatty Acids (Omega-3)	Eicosapentaenoic acid (EPA)	20:5ω-3	Fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
	Docosapentaenoic acid (DPA)	22:5ω-3	Fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
	Docosahexaenoic acid (DHA)	22:6ω-3	Fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
Polyunsaturated Fatty Acids (Omega-6)	Linoleic acid	18:2ω-6	Most vegetable oils (included walnut oil)
	Ɣ-linolenic acid	18:3ω-6	Evening primrose, borage and blackcurrant seed oils
	Arachidonic acid	20:4ω-6	Animal fats, liver, egg lipids, fish

3. Effects of Fatty Acids and Walnut on Health and Nutrition

Fatty acids are considered the crucial nutrients that affect growth and development and nutrition-related chronic disease later in life (Anonymous, 2018). They are major components of cell membrane structure, modulate gene transcription, function as cytokine precursors, and serve as energy sources in complex, interconnected systems (Norris and Milton, 2013). That is, fatty acids are required in human nutrition as a source of energy, and for metabolic and structural activities. The most common dietary fatty acids have been subdivided into three classes as saturated, monounsaturated, and polyunsaturated fatty acids.

The energy provided by saturated fatty acids is equivalent to the calorie given by other fatty acids, but they cause fat accumulation and gaining weight in the body. It is stated that tm he consumption of saturated fat should be reduced to minimize the risk of cardiovascular diseases. Saturated fatty acids prevent the clearance of LDL in the blood and increase the rate of fat in the blood. As a result, they can cause atherosclerosis by forming deposits in the vessels. It is also stated that it increases the level of LDL cholesterol and leads to the formation of insulin resistance, thus increasing the tendency to diabetes (Çakmakçı and Tahmas-Kahyaoğlu, 2012; Siri-Tarino et al., 2010). The effects of monounsaturated fatty acids on LDL cholesterol and triglycerides are neutral, but they have an increaser effect on the high-density lipoprotein (HDL) cholesterol. A significant reduction in the level of LDL cholesterol can be achieved by consuming polyunsaturated fats. There are two main groups of polyunsaturated fatty acids; Omega-3 and omega-6 fatty acids. Omega-3 fatty acids are found in oily marine fish as well as the oils of flaxseed, canola, soy, walnut, and hazelnut. Omega-3 fatty acids are found in most vegetable oils, including walnuts. Omega-3 fatty acids reduce blood triglyceride levels by reducing the production of LDL cholesterol. Due to the heart-protective effect of omega-3 fatty acids, people who consume these oils have a decrease in deaths due to coronary heart disease (Samur, 2012; Siri-Tarino et al., 2010; Maguire et al., 2004).

Among polyunsaturated fatty acids, arachidonic acid, α-linolenic acid, and linoleic acid are significant and essential fatty acids in terms of health and nutrition by playing substantial roles in biochemical and physiological events in the body. Omega fatty acids, which are a type of polyunsaturated fatty acid, have been associated with various health benefits like the treatment of rheumatoid arthritis and coronary heart disease, improving blood pressure control, and preserving kidney function. The effects of omega-3 fatty acids on various disorders such as cancer, edema, rheumatoid arthritis, cardiovascular, are closely related to their metabolism. Therefore for the protection against metabolic diseases and disorders, the inclusion of omega-3 fatty acids in the diet has been widely accepted as one of the keystones of a healthy lifestyle and nutrition (Gogus and Smith, 2010; Rennie et al., 2003; Holm et al., 2001; Çakmakçı and Tahmas-Kahyaoğlu, 2012).

Essential fatty acids, such as omega-3 and omega-6 fatty acids, serve crucial cellular functions. For example, during pregnancy and lactation, omega-3 essential fatty acids are accepted as structural components for the development of the brain and central nervous system. They are a necessary part of the human diet because the body can't synthesize these molecules. Essential fatty acids may affect the prevalence and severity of cardiovascular disease, diabetes, cancer, and age-related functional decline (White, 2009; Anonymous, 2018).

Walnut is considered important nutrition with benefits for human health because walnut kernels are rich in protein, oil, unsaturated fatty acids, vitami ns, minerals, essential fatty acids, and other nutrients.

Walnut oil is a good source of omega-3 and omega-6 polyunsaturated fatty acids, which are reported to have beneficial effects on blood lipids, by lowering blood cholesterol, decreasing the rate of serum concentrations of LDL, and increasing HDL. Compared with other nuts, walnuts have the lowest ratio of saturated fatty acids to total fatty acids (Geng et al., 2021; Nogales-Bueno et al., 2021; Kafkas et al., 2020; Li et al., 2007). The major fatty acids found in walnut oil are oleic acid, linoleic acid, and α-linolenic acid (Rabrenovic et al., 2011).

In the study carried out by Nergiz-Ünal et al. (2013), it was remarked that the atherosclerotic plaque development in the aortic arch of mice fed with walnuts was a 55% reduction. Plasma levels of triglycerides, cholesterol, and prothrombin also lowered by 36%, 23%, and 21%, respectively, compared to the control diet. In addition, the accumulation of lipids in the liver was decreased, while plasma antioxidant capacity was increased.

In a study in hypercholesterolemic patients, it was assessed the effects on serum lipids and cardiovascular risk of replacing 40% of the fat in a normal diet with olive oil, walnuts, or almonds. In the 18 participants who joined the study, LDL-cholesterol was reduced from baseline by 7.3%, 10.8%, and 13.4% after the olive oil, walnut, and almond diets, respectively. Total cholesterol and LDL/HDL ratios decreased in parallel (Damasceno et al., 2011).

Torabian et al. (2010) investigated the effect on the total cholesterol and triglyceride levels of long-term walnut consumption in 87 subjects with normal to moderate high plasma total cholesterol. The results showed that including walnuts as part of a normal diet favorably altered the plasma lipid profile. The lipid-lowering effects of walnuts were more evident among subjects with high plasma total cholesterol.

The fact that walnut is nutritionally valuable food comes from their rich composition, especially their polyunsaturated fatty acid profile.

4. Analysis of Fatty Acids

The determination of fatty acids in oil-containing samples is carried out in three stages (Anonymous, 2018):

4.1. Extraction of oil from samples: Samples are ground before the solvent extraction to produce a more homogeneous sample and to increase the surface area of lipid exposed to the solvent. For polar lipids such as glycolipids or phospholipids, it is suitable for polar organic solvents such as alcohols. On the other hand, it is suitable the nonpolar solvents such as hexane, ethyl ether, petroleum ether, and pentane for nonpolar lipids. In samples such as walnuts, peanuts, almonds, peanuts, and hazelnuts, the soxhlet method is generally used (Hewavitharana et al., 2020; Servaes et al., 2015; Señoráns and Luna, 2012; Min and Ellefson, 2010; Thiex et al., 2003).

4.2. Preparation of ester derivatives of fatty acids: The fatty acid composition of samples is determined as the methyl esters of fatty acids by GC. The conversion process of fatty acids into fatty acids methyl esters is called derivatization. Acid and basic derivatization methods are used in this process. The commonly used acid derivatization reagents are hydrochloric acid, acetyl chloride, sulfuric acid, and boron trifluoride. In basic derivatization methods, reagents such as sodium methoxide, sodium bisulfate, and potassium hydroxide are used. There are also other methods used reagents such as trimethylsulfonium hydroxide and pentafluorobenzyl bromide (Avci et al., 2018; Asperger et al., 2001; Ichihara and Fukubayashi, 2010; Aldai et al., 2005; Ostermann et al., 2014; Hewavitharana et al., 2020).

4.3. Analysis by gas chromatography (GC): Analyses of fatty acids methyl esters are performed by GC having a flame ionization detector. For analyses, it is preferred bonded polar capillary columns (Anonymous, 2018).

5. Fatty Acids in Wlanut Oil

Many studies have been conducted to determine the fatty acid composition of different walnut cultivars/genotypes. The results compiled from these studies are given in Table 3. Palmitic acid (C16:0),

Tablo 3. Fatty Acid Composition of Different Walnut Cultivars/Genotypes

Cultivar Genotype	Origin	Fatty Acids												References
		C14:0	C16:0	C16:1ω7	C18:0	C18:1ω9	C18:1ω7	C18:2ω6	C20:0	C18:3ω6	C20:1ω9	C18:3ω3	C21:0	
Uşak	Uşak Turkey	0,03	6,36	0,05	2,62	17,58		59,32			0,13	13,75		Yildiz et al., 2021
Adilcevaz	Van Lake Turkey		4,30		1,00	29,20		55,80				9,90		Batun et al., 2017
Ahlat			5,10		1,00	29,80		52,00				12,70		
Edremit			5,90		2,40	29,80		52,90				9,70		
Çatak			5,70		1,60	24,30		55,50				13,10		
KW1-50	Kayseri Turkey		5,71		2,12	25,44		54,40				12,30		Uzun et al., 2021
W1-4	Turkey	0,13	5,73		2,24	23,91		52,13				15,94		Dogan and Akgul, 2005
44HEK	Malatya Turkey		5,57		2,87	33,63		47,12				10,17		Gerçekçioğlu et al., 2020
Kaman-2	Kırşehir Turkey		6,30		2,60	20,50		55,50				14,80		Özcan et al., 2010
Kaman-5			6,50		2,60	26,40		53,60				14,30		
Büyük Oba			6,30		2,50	22,20		49,70				14,50		
Kaplan-86	Hatay Turkey		7,21		4,49	28,01		50,31				9,75		Bayazıt and Sümbül, 2012
Malatya-1			6,98		3,22	19,33		59,89				9,97		
Şebin			7,13		3,82	34,01		46,55				8,44		
Şen 1			7,24		3,72	27,49		51,52				9,96		
Tokat 1			7,70		3,67	21,09		56,45				11,00		
KR 2			7,28		3,73	34,03		45,30				9,66		
77H1			8,77		3,74	22,45		55,23				9,79		
65/4			7,14		4,11	36,76		41,55				10,10		
Kaplan-86	Adana Turkey	0,07	6,22	0,04	3,25	19,07		58,64				12,70		Ada et al., 2021
Bilecik		0,06	6,53	0,04	3,17	21,95		56,64				11,63		
Yalova-1		0,05	6,07	0,04	2,25	18,82		62,51				10,26		
Yalova-3		0,06	6,21	0,09	2,51	25,51		52,13				11,81		
Yalova-4		0,06	6,84		1,96	27,09		54,22				9,83		

Cultivar Genotype	Origin	Fatty Acids												References
		C14:0	C16:0	C16:1ω7	C18:0	C18:1ω9	C18:1ω7	C18:2ω6	C20:0	C18:3ω6	C20:1ω9	C18:3ω3	C21:0	
Chandler	Kahramanmaraş Turkey	0,04	7,63		1,77									Arcan., 2021
Kaplan-86		0,62	7,44		1,92									
Bilecik	Adana Turkey	0,02	5,82	0,10	3,82	13,80		62,92	0,12			13,16		Kafkas et al., 2017
Chandler		0,02	5,92	0,12	3,41	14,47		62,82	0,10			12,92		
Hartley		0,02	6,16	0,11	3,59	12,95		64,56	0,12			12,12		
Howard		0,02	6,13	0,11	3,44	14,36		62,20	0,12			13,26		
Maraş-12		0,02	6,57	0,13	3,70	21,02		59,62	0,11			8,55		
Maraş-18		0,02	6,98	0,13	3,59	27,57		53,42	0,10			7,83		
Midland		0,02	6,41	0,12	3,86	17,63		61,22	0,11			10,33		
Pedro		0,02	6,72	0,11	3,97	14,77		61,89	0,13			12,17		
Şen		0,03	6,58	0,12	3,23	26,86		53,24	0,11			9,50		
Serr		0,02	6,57	0,12	3,88	14,94		61,37	0,10			12,74		
Franquette	Portugal	0,03	7,48	0,05	2,43	16,99	1,53	59,22	0,07	0,06	0,19	11,69		Amaral et al., 2003
Marbot		0,02	7,14	0,08	2,77	16,51	1,26	58,90	0,08	0,07	0,19	12,74		
Mayette		0,03	7,00	0,08	2,55	18,09	1,21	57,46	0,07	0,05	0,19	12,98		
Melanaise		0,03	7,02	0,07	2,65	14,49	1,24	61,31	0,06	0,04	0,17	12,51		
Lara		0,03	6,94	0,06	2,22	14,26	1,29	62,50	0,06	0,05	0,18	12,16		
Parisienne		0,03	6,32	0,07	2,41	17,45	1,11	62,45	0,07	0,03	0,22	9,64		
Chandler	Spain		5,96	0,05	2,28	12,98	1,08	62,22		0,17	0,14	14,23	0,57	Nogales-Bueno et al., 2021
Franquette			7,17	0,07	2,40	14,84	1,47	58,64		0,18	0,14	14,12	0,54	
Howard			6,88	0,05	2,25	12,43	0,99	60,36		0,14	0,12	16,92	0,56	
Lara			6,76	0,06	2,38	12,16	1,01	63,74		0,18	0,17	12,61	0,58	
Tulare			6,65	0,06	2,47	12,27	0,93	62,27		0,09	0,10	14,60	0,26	
Sampion	Serbia		7,10	0,40	1,60	19,00		60,90				11,00		Rabrenovic et al., 2011
Jupiter			7,00	0,10	1,80	22,90		58,10				9,90		
Sejnovo			6,70		1,70	16,20		63,30	0,80			11,20		
Elit			7,10	0,30	2,20	21,60		58,80				9,90		
G-139			7,70	0,40	1,60	19,80		57,20				13,60		

Cultivar Genotype	Origin	Fatty Acids												References
		C14:0	C16:0	C16:1ω7	C18:0	C18:1ω9	C18:1ω7	C18:2ω6	C20:0	C18:3ω6	C20:1ω9	C18:3ω3	C21:0	
Serbia	Serbia		7,03	0,11	2,75	14,47	1,34	63,15				11,15		Petrović-Oggiano et al., 2020
Esterhazy			7,47		1,63	17,44	0,68	58,83			0,12	13,54		
G139			6,65		1,40	16,46	0,71	61,98			0,14	12,71		
G120	Europe and United Stadtes		7,73		2,05	19,58	0,69	57,09			0,12	12,45		
Tehama			7,61		1,35	19,54	0,81	57,88			0,14	12,38		
Vina			6,46		1,43	17,94	0,68	58,03			0,11	15,07		
Rex			6,59		0,07	12,66	0,81	62,48			0,11	16,17		
Dublin's Glory			7,76		0,08	18,95	0,85	57,01			0,12	13,10		Savage et al., 1999
Meyric			7,30		0,08	18,09	0,85	58,43			0,11	13,31		
Stanley	New Zealand		6,72		0,08	20,36	0,63	59,24			0,11	11,18		
McKinster			6,22		0,06	18,71	0,77	61,31			0,06	10,65		
150			7,15		0,06	17,39	0,74	60,45			0,12	12,65		
151			6,75		0,06	16,20	0,71	61,72			0,14	12,71		
153			6,84		0,06	14,35	0,58	61,64			0,10	15,21		
Ivanhoe		0,04	7,86	0,24	2,43	16,95		59,23				13,24		
Franquette		0,06	7,83	0,08	2,04	14,17		64,26				11,57		
Howard		0,04	5,74	0,06	2,59	11,36		64,34				15,87		
Durham		0,03	6,87	0,13	2,39	20,65		60,23				9,70		
Earliest		0,04	6,95	0,13	2,40	17,49		63,11				9,88		
Solano		0,02	9,49	0,14	2,27	10,85		61,50	0,08			15,65		
Hartley	USA	0,06	7,09	0,13	2,50	13,74		65,26	0,12			11,10		Kafkas et al., 2020
R. Livermore		0,03	8,03	0,13	2,27	18,34		61,69	0,06			9,46		
Chandler		0,02	6,46	0,07	2,92	17,48		58,96	0,06			14,03		
95-014-3		0,02	7,16	0,46	2,40	15,99		60,83				13,13		
03-001-2357		0,05	6,73	0,24	2,45	14,31		60,00	0,08			16,14		
R. Livermore		0,01	6,59	0,12	2,15	14,78		64,31	0,02			12,01		
Chenier			6,95	0,08	3,00	19,21		61,67				9,08		
Sinensis#5			7,16	0,14	2,44	15,71		66,07	0,08			8,41		

Cultivar Genotype	Origin	Fatty Acids												References
		C14:0	C16:0	C16:1ω7	C18:0	C18:1ω9	C18:1ω7	C18:2ω6	C20:0	C18:3ω6	C20:1ω9	C18:3ω3	C21:0	
Aramoko-Ekiti	Nigeria	0,08	12,19	0,36	5,08			72,87	0,10			9,09		Ogungbenle and Anisulowo, 2014
NI	New Zealand		7,70		2,20	20,80		58,00				11,10		Chisholm et al., 1998
Cork	Ireland	0,13	6,70	0,23	2,27	21,00		57,46	0,08			11,58		Maguire et al., 2004
Combe	Canada	0,03	5,87	0,06	3,24	15,73		57,29	0,11			15,75	0,02	Li et al., 2007
Lake		0,03	5,59	0,05	2,83	16,39		60,96	0,05			12,11	0,02	
Min. value		0,01	4,30	0,04	0,06	10,85	0,58	41,55	0,02	0,03	0,06	7,83	0,02	
Max. value		0,62	12,19	0,46	5,08	36,76	1,53	72,87	0,80	0,18	0,22	16,92	0,58	
Avg. value		0,05	6,85	0,13	2,40	9,46	0,96	58,64	0,11	0,10	0,14	12,04	0,36	

stearic acid (C18:0), oleic acid (C18:1ω9), linoleic acid (C18:2ω6), and α-linolenic (18:3ω3) are seen to be dominant fatty acids in walnuts grown in Turkey's ecology. Walnuts grown in European ecology contain *cis*-vaccenic acid (C18:1ω7) around a 1% rate in addition to these fatty acids. According to the data in Table 3, it is seen that the walnut genotype grown in Nigeria contains the highest levels of C16:0, C18:0, and C18:2ω6. It can be said that growing conditions and cultivar characteristics are effective in the variation between the results. For example, it has ranged from C16:0 between 4.30% and 12.19%, C18:0 0.06% and 50.8%, C18:1ω9 10.85% and 36.76%, and C18:2ω6 41.55% and 72.87% (Tablo 3).

The total of unsaturated fatty acids (average %81,47) in walnut oil has a higher value than the total of saturated fatty acids (average %9,76). Containing high levels of alpha-linolenic and linoleic acids, which are essential fatty acids known as omega-3 and omega-6, make walnut an indispensable food. As shown in Table 3, the average levels of omega-

3 (C18:3ω3) and omega-6 (C18:2ω6) fatty acids are 12.04% and 58.64%, respectively. Compared with most other nuts, walnuts are highly enriched in omega-6 and omega-3 polyunsaturated fatty acids, which are essential dietary fatty acids. Essential fatty acids also make substantial contributions to the prevention of cardiovascular diseases. It has importance to include walnuts in the normal diet, which is similar to the shape of the human brain and is thought to contribute greatly to brain development.

6. Conclusion

The importance of natural nutritional supplements in the protection of our health has increased in recent years, with scientific studies proving the effectiveness of some foods in the prevention and treatment of diseases naturally. This situation has increased the demand for natural health products.

Walnut, which is in the group of nuts and contains a high level of oil, is considered a functional food due to its nutritious and beneficial effects on health. It contains abundantly the essential fatty acids, which are effective in the prevention of many diseases, especially cardiovascular diseases, and in brain development. It should not be an exaggeration to say that one of the elements of a healthy and balanced diet is walnuts.

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Part XV

Nature's Miracle Walnut and Human Health

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INTRODUCTION

The history of using plants to treat human health and many diseases is as old as human history; such that it has a history of several thousand years in many countrie. In the past centuries, natural remedies, especially medicinal plants, were accepted as the basis of treatment. In the last decade, it has been shown that there is a great tendency and willingness to accept natural treatments in developed and developing countries (Delaviz et al., 2017). The use of plants in traditional medicine for many diseases has become widespread in the world. In many studies conducted over the last 30 years; They found that there is a relationship between people's eating habits and diseases. While investigating the relationships between some well-known diseases and nutrition, the most questioned food component/food item was fats.

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Walnut, a member of the Juglandaceae plant family, is one of the most valuable and common tree nuts worldwide due to its rich nutritional content. Thanks to its high adaptability to different climatic conditions that walnuts are widely cultivated, especially in regions with semi-tropical or temperate climates (Kafkas et al., 2020). The walnut which has a history of thousands of years, is the oldest tree known to man and is used in the treatment of different diseases. Almost all parts of this plant such as bark, kernel (seed), flower, leaf, green bark, septum, oil have unique food, cosmetic pharmaceutical industries and medicinal properties (Figure 1 and 2) (Vadivel et al., 2012; Gupta et al., 2019). Therefore, the different tissues of the walnut tree, besides the antioxidant and antimicrobial effects of the different compounds they contain, as well as the inflammatory antihistamine, antiseptic, antiulcer, antinociceptive, antidiabetic, antiasthmatic, antifertility, central nervous system, wound healing, lipolytic, insecticide, larvicide, and human health effects. contains many features (Zang et al., 2018; Salejda, et al., 2016; Trandafir, et al., 2016; Zarghami Moghaddam et al, 2017; Raja et al., 2017; Jahanban-Esfahlan et al., 2019). Furthermore, Walnut (Juglans regia L.) has long been consumed as a highly nutritious food in many parts of the world and is an important component of the Mediterranean diet. Walnuts are widely available in our diet and have long been known for their nutritious properties. Walnuts are rich in unsaturated fatty acids (omega 3), fiber, protein sources, vitamins (vitamin A, vitamin E, vitamin K, vitamin B1, vitamin B2, vitamin B6, folic acid and niacin), various minerals (copper, manganese, calcium, phosphorus, zinc, magnesium, sodium, potassium, selenium, iron) iodine and polyphenols that It is a good diet and antioxidant source containing (Sen, 2011; Şen ve Karadeniz, 2015; Chatrabnous ve ark., 2018; Gulsoy et al., 2021). In recent years, in many studies conducted in different organs, various studies have been carried out on the determination and determination of biochemical, phytochemical and antioxidant properties of walnuts and their contributions to human nutrition and health (Akca, 2001; Simsek and Gülsoy, 2016; Binici et al., 2021. It has been determined that these substances in walnuts have protective and curative effects in many diseases as a result of both epidemiological and clinical studies in terms of human health. these diseases are diabetes, cancer, cardiovascular diseases, inflammation and neurodegenerative effects that cognitive function, heart health, cancer, diabetes, weight, gut health, and reproductive health. Its many disease-promoting properties and potential health effects has been attributed to the presence of many different bioactive nutrients, including mainly unsaturated fatty acids, fiber, protein, phenolic compounds, tocopherols and many minerals. This review aimed to explore potential roles in various health outcomes, including heart health, diabetes, cancer, cognitive function, brain, and obesity (weight)

Health Benefits of Walnut

The benefits of walnuts, which are easily accessible, can be stored for a long time, can be added to foods in different ways and are easy to add to the daily diet, are endless. Walnut, which is a part of our daily life, benefits human health in many ways when consumed regularly and consciously. Thanks to the many bioactive compounds it contains, walnuts are becoming more popular with each passing day and are more and more involved in human nutrition, thanks to their nutritional value and strong antioxidant level, their important role against oxidative stress and the degenerative effects of free radicals. Therefore many researchers have attached great importance to determining the bioactive compound content of walnut and other plant groups, evaluating the antioxidant activity, and the importance of these substances in terms of human health

Walnut and Heart Health

Cardiovascular diseases are one of the most common diseases and the most important causes of death worldwide, and still a danger to humans. Heart disease - or cardiovascular disease - is a broad term for chronic conditions related to the heart and blood vessels. Among these terms, tachycardia, heart attack, heart failure, arrhythmia, heart valve disease and heart failure are the most known ones. High LDL (bad cholesterol) is the main cause of heart disease (Zambon, et al., 2000; Anderson et al., 2001; Spaccarotella, et al., 2008; Guasch-Ferré, et al., 2013). Epidemiological and clinical studies conducted in recent years have shown that there are strong relationships in reducing cardiovascular diseases and increasing the antioxidant defense system, especially thanks to the characteristic lipid profile of walnuts, which is one of the nut groups. Omega 3 fatty acids are seen as benefits for improving heart health. Thanks to the omega 3 fatty acids in walnuts, it helps to protect heart health. In addition, thanks to gamma-tocopherol, a type of vitamin E, and other elements, the elements play an important role in cleaning the environment of the heart, especially cholesterol, and reducing the risk of cardiovascular disease by reducing insulin resistance, cholesterol concentrations, lipid peroxidation and oxidative (Rim et al., 1993; Kris-Etherton, et al., 1999; Anderson, et al., 2001; Albert, 2002; Spaccarotella, et al., 2008; Banel et al., 2009; Ros, 2009; Bernsteine, et al., 2010; Kendall et al., 2010; Ros, 2010; Sabate et al., 2010; Estruch et al., 2013; Guasch-Ferré, et al., 2013; Aune et al., 2016; Ros ve ark., 2018). However, some researchers have reported in recent studies on walnuts that ellagitannins in walnuts have protective effects in relation to cardiovascular diseases (Papoutsi et al., 2008; Spaccarotella et al., 2008; Larrosa et al., 2010; Vadivel et al., 2012; Nergiz-Unal et al., 2013; Sánchez-González, et al., 2017). There is growing evidence and studies are ongoing regarding the role of walnut consumption in reducing the risk of coronary heart disease.

Walnut and Cancer

Nowadays, while cancer continues to be one of the most important health problems all over the world, the rapid increase of this disease, which results in death in the world, is very important for human health. (Anand et al., 2008; Globocan, 2012; Cevik and Pirinçci, 2017). It is not correct to say that walnuts cure cancer, but there are opinions that they can eliminate the factors that cause cancer or reduce the risk of developing the disease. Some researchers have widely investigated the effect of polyphenols in walnuts and especially ellagitannins on the initiation, development, progression and prevention of cancer and found positive results (Owen et al., 2000; Clemons, et al., 2001; Corona et al., 2007; Spaccarotella et al., 2008; Khan and Mukhtar, 2013; Reiter et al., 2013; Hardman, 2014; Sanchez-Gonz alez et al., 2015). According to different sources, the relationship between cancer and nutrition varies between 10-70%, with an average of 35% (Cevik and Pircisci, 2017). In recent years, researchers have tried to find out the relationship between nutrition and cancer and which foods have protective effects against cancer, and studies are still ongoing. Some studies have reported that walnuts have the highest antioxidant activity and can play a beneficial role in the prevention of cancer and are associated with a decrease in deaths due to this disease. (Thiebau et al., 2009; Heuvel et al., 2012; Guasch-Ferré et al., 2013; Aune et al., 2016; Ozkan and Celik, 2016; Fang et al., 2021). A study led by Mantzoros 2015 showed that a diet containing walnuts could slow colorectal tumor growth by exerting beneficial effects on cancer genes. This is the first study to evaluate whether walnut consumption causes changes on micro-ribonucleic acids (mRNA), which are defined as nucleotides that play a role in gene expression. The researcher stated that the walnut-containing diet contains fatty acids that protect against colon tumor due to the direct effects or the additive effect or synergistic effects of other multiple components in walnuts. The investigator found that the total amount of omega-3 fatty acids, including plant-based alpha-linolenic acid (ALA), was 10 times higher in tumor tissues of mice fed a walnut-containing diet compared to mice fed a control diet, particularly the smaller tumor size had higher omega-3 fatty acids in tumor tissues. shows that it is related to the percentage of fatty acids; This suggests that ALA may provide a protective benefit. Tumor growth rate was also observed significantly slower in the walnut group compared to the control group (Mantzoros, 2015). In another study, Ros et al., (2018), found that walnuts contain substances such as some polyphenols, phytomelatonin, ellagitannins and urolithin that enable them to have anti-cancer effects. In addition, it states that it reduces substances that cause cancer, thanks to an important form of vitamin E in its content. Walnuts in a study investigating the effects of gamma-tocopherol on factors related to prostate and vascular health, especially in elderly men, it was reported that walnuts may have an effect on prostate health due to the antioxidant vitamin E content (Spaccarotella et al., 2008). In some studies on breast cancer, which is common in women, it has been stated that walnut consumption may suppress the growth and survival of breast cancer in humans (Brennan et al., 2010; Nagel et al., 2012; Kim et al., 2014; Hardman et al., 2019). It is also known that omega-3 fatty acids containing alpha-linolenic acid have a protective effect on different types of cancer such as bladder, breast and endometrial cancer. In a study by of common breast cancer in women, it was noted that walnut consumption may suppress the growth and survival of breast cancer in humans. In walnut oil, besides alpha-linolenic acid; There are also other fatty acids that may have anticarcinogenic effects, such as oleic, linoleic, palmitic and stearic acid (Batirel et al., 2018). However, it is seen that more research needs to be done in order to better understand the effect levels of cancer and nutrition (walnut) in humans.

Brain and Walnut

Population growth in the world is increasing and increasing life expectancy is causing a large population to age. It is seen that this demographic change has increased the incidence of neurodegenerative diseases in terms of human health. Neurodegenerative diseases are defined as the damage of nerve cells or the failure of their functions to fulfill their functions and deterioration (Marx, 2006; Poulose, et al., 2014; Jack ve ark., 2015). Although the central nervous system is particularly vulnerable, it also affects other organ systems (heart disease, cancer, arthritis, diabetes and age-related disorders). Therefore, systemic protection from oxidative stress and inflammation may affect the brain not only from their direct effects, but also in the functioning and protection of other organs. In many of these neurodegenerative diseases, people continue their negative and irregular life activities (such as lifestyle, unconscious and unhealthy diet, lack of sports, smoking). One of the most effective and cheapest ways to prevent this situation can be obtained with a healthy diet with antioxidant and anti-inflammatory effects. Walnut (*Juglans regia* L.), which is the most important type of nuts, has been determined in many studies to be rich in high amounts of nutrients and bioactive compounds, including Omega-3, polyphenolics, polyunsaturated fatty acids (alpha-linolenic acid, linoleic acid (Pereira, et al., 2008; Shukitt-Hale, et al., 2008; Willis, et al., 2009a,b,c,d; Poulose, et al., 2014;

Câmara, et al., 2016; Pribis, 2016). In various clinical studies, PUFAs and other phytochemical compounds play an important role in brain health and healthy neuronal processes. The important alpha-linolenic acid, which is responsible for the modulation of membrane stability, neuroplasticity, signal transmission rate, and serotonin and dopamine concentrations found in walnuts, has been shown to directly affect the physiology of the brain by affecting different parts of the brain. Alpha-linolenic acid is also a precursor of a fatty acid that regulates serotonin and dopamine density. (Pereira et al., 2008; Heinrichs, 2010; Poulose ve ark., 2014; Arab ve Ang, 2015; Jack et al., 2015; Pribis, 2016). Pribis, (2016) reported that people showed positive effects on mood (anger, anxiety, fear, depression and confusion) and reduced these problems. The result, there is growing evidence that walnuts have beneficial effects on the brain and mood, thanks to the many bioactive compounds they contain.

Diabetes and Walnut

Diabetes is one of the most common and rapidly increasing diseases in the world, which is at the forefront among the diseases of the age, which is the primary cause of the formation of many different important diseases. Type 2 diabetes is the most common type of diabetes, which has many different types. This disease is basically defined as an imbalance between the body's blood sugar and the hormone insulin, which ensures the proper use of this sugar in the cells. Therefore, it is of great importance to keep the blood sugar in the body under control in order to prevent Diabetes (Pan, et al., 2013;

Nijike et al., 2015; Fang et al., 2016; Ros, et al., 2018; Cole and Florez, 2020). In order for people's vital activities to continue without any problems, and especially for the prevention and protection of diabetes, people should pay attention to their lifestyle and proper eating habits. Walnuts contain high amounts of dietary fiber, antioxidants and phytosterols. Compared to other nuts containing high amount of monounsaturated fat, walnuts are very rich in Polyunsaturated fatty acids (PUFAs) compared to other types. (Maguire et al., 2004; Chen and Blumberg, 2008; Anonim, 2021). In particular, walnuts, due to their fatty acid compositions (linoleic acid and α-linolenic acid), have been shown to positively affect insulin resistance and type 2 diabetes risk by increasing circulating PUFA concentrations (Riserus, 2008; Riserus, 2009; Zibaeenezhad ve ark., 2016Arab ve ark., 2018; Liu ve ark., 2020). Walnut have monounsaturated fatty acids, protein and fiber and are low in carbohydrates. This means they help fill us up while keeping blood sugar low. Some epidomological studies have found that the effects of walnut consumption on insulin secretion (Estruch et al., 2006) and glucose homeostasis (Jenkins et al., 208), causing an inverse relationship between them, are associated with a lower risk of type 2 diabetes (Pan et al., 2013). A recent study published in Circulation Research found a lower risk of heart disease and death in people with Type 2 diabetes who ate nuts. In addition, in many studies, walnuts have been shown to improve good cholesterol and reduce bad cholesterol.

Walnut ve Diet quality (Obesity)

According to the World Health Organization (DSO), obesity is an important disease and a public health problem that is difficult to treat and occurs as a result of an increase in tissue as a result of excessive accumulation of fat in the body. The reason that makes obesity important is not only about appearance, but also it is known to be associated with many diseases (diabetes, cardiovascular disease, cancers, musculoskeletal disorders). Today, Obesity is increasing day by day both in the developed and developing world (Koruk and Şahin, 2005; Brennan, et al., 2010; Çayır et al., 2011; Zileli et al., 2016; Njike, et al. , 2017; Liu et al., 2019; Fang et al., 2020). Nutritional habits of people have an important role in the development of obesity. It is an important component of hard-shelled foods, especially walnut diet. Walnut, "Obesity’’ is known to be associated with various diseases, and some studies have also stated that it has the potential to prevent obesity and has an effect. (Estruch et al., 2018; Pal, 2020). Brennan et al., (2010) reported that walnuts good fat (ALA/omega 3), fiber and protein, especially pulp, can help in weight management, especially providing satiety and reducing calorie consumption. In another study, it was seen that it may have a different fatty acid metabolism in terms of polyunsaturated fatty acids and saturated fatty acids, so it will affect weight loss (Fang et al., 2020). In another study, Katz et al., (2012) has reported that A meta-analysis investigating the effect of walnut consumption on blood lipids found that during short-term trials, walnut-enriched diets significantly reduced total cholesterol and low-density lipoprotein cholesterol (LDL-C) compared with control diets. As a result, one of the most important strategies to reduce the incidence of obesity in many epidemiological studies is to address an optimal energy balance. For this reason, although walnut is a fatty acid-rich food, it has been shown that there is a relationship between daily and regular walnut consumption and weight loss. (Brennan et al., 2010; Jackson and Hu, 2014; Njike ve ark., 2015; Njike, et al., 2017; Rock, et al., 2017; Bitok, et al., 2018; Fang, et al., 2020)

CONCLUSION

Walnut, which is the most important type of hard-shelled fruit, has a very important place as a support in nutrition and medical treatments in terms of many bioactive compounds it contains. In this study, it gives brief and important information about walnuts and human health. In recent years, some studies on walnuts and their bioactive components and diseases have been examined. Studies have shown that walnuts are rich in unsaturated fatty acids such as linoleic acid and Omega 3, protein, vitamins and minerals, as well as bioactive substances such as melatonin, ellagic acid, vitamin E, carotenoids and polyphenols, which have antioxidant effects. Many epidemiological and clinical studies conducted in recent years have shown that many epidemiological and clinical studies conducted regularly in recent years have shown that regular walnut consumption is associated with coronary heart disease, brain, hypertension, diabetes, weight gain over time, obesity, visceral obesity, metabolic Syndrome has shown to have a beneficial effect on many health outcomes, such as cancer. It has been shown that walnut consumption has an important place in nutrition and prevention of diseases such as coronary heart disease, brain, hypertension, diabetes, weight gain over time, obesity, cancer. In conclusion, walnut is an optimal healthy food for a balanced and healthy diet. For this reason, it is necessary to raise awareness about many beneficial bioactive compounds in walnuts and their benefits, scientific research on their benefits to human health should be increased and studies on different diseases should continue. For a long health life, walnuts should be included in people's healthy and regular nutrition programs.

Figure 1. Evaluation of different organs of walnut

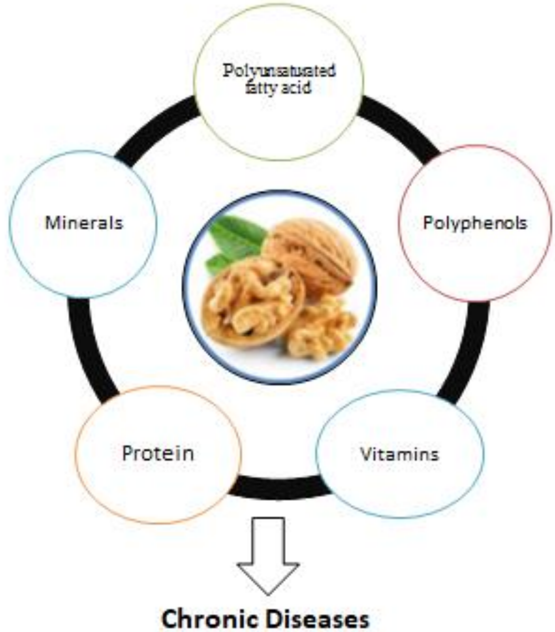

Figure 2. Walnut bioactive compounds and health

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