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# Genetic Diversity and Breeding of Persimmon

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## Abstract

The genus *Diospyros*, which is distributed in tropical and subtropical regions of the world, contains hundreds of plant species. However, four species of them have commercial importance. *D. kaki* Thunb. is the most widely cultivated species of the *Diospyros* genus. Persimmon (*D. kaki* Thunb.) is grown in many parts of the world that display subtropical climate conditions. In recent years, the cultivation of persimmon has found renewed interest in various countries of the Mediterranean basin. In China (which is the origin of persimmon) and in Japan and Korea (where it is grown widely), persimmons were selected from some well-known old varieties. Recently in countries such as Italy, Spain, USA, Brazil, Turkey and Israel, persimmons were selected from new cultivars. Currently China, Japan and Korea have the big persimmon germplasm collections with a large number of varieties and other *Diospyros* species. Also, Italy, Spain, USA, Brazil, Turkey, Israel, Azerbaijan, Uzbekistan and Pakistan have constituted the germplasms by high commercial value cultivars and/or local varieties. In this chapter, we tried to provide an overview of the genetic diversity and breeding of persimmon by combining literature with our studies.

**Keywords:** *Diospyros*, genetic resources, selection, hybridization, breeding

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## 1. Introduction

Persimmon is fleshy fibrous subtropical and tropical, deciduous fruit belonging to *Ebenaceae* family. The Oriental persimmon (*Diospyros kaki* Thunb.) is an exotic fruit rich in vitamins, nutrients and antioxidants vital for optimum health with various medicinal and chemical uses of fruits and leaves. Its fruit is usually consumed as a fresh or dried fruit. It is believed to have originated in the mountain area of southern China and has been cultivated as an important fruit crop in China, Korea as well as in Japan for centuries [1]. It is commonly

cultivated in warm regions of the world including China, Korea, Japan, Brazil, Spain, Turkey, Italy and Israel. The world's total persimmon production was low between 1961 (990,079 tons) and 1993 (1,290,971 tons). However, persimmon production has increased since 1995. Total persimmon production of the world was 5,190,624 tons in 2014 (**Table 1**). Similarly, while persimmon acreage was low from 1961 to 1993, the acreage expanded rapidly increasing from 262,039 ha in 1993 to 1,028,051 ha in 2014 [2].

As represented in **Table 2**, China is the highest producer with a production of about 3,730,800 tonnes. This amount of production accounts for about 71.88% of the world's production. It is followed by Korea (428,363 tonnes), Spain (245,000 tonnes), Japan (240,600 tonnes), Brazil (182,290 tonnes), Azerbaijan (140,405 tonnes), Taiwan (72,674 tonnes), Uzbekistan (66,000 tonnes), Italy (39,149 tonnes), Israel (36,592 tonnes) and Turkey (33,470 tonnes) [2, 3]. The variation of production amounts of the countries differed by years. As is presented in **Table 2**, China's persimmon production has steadily increased from 495,000 tonnes in 1961 to 3,730,800 tonnes in 2014. Similarly, Korea's production increased by 32.28 times and has reached 428,363 tonnes. Spain has shown the maximum rate of increase with the production increasing from 591 tonnes in 1991 to 245,000 tonnes in 2014. In Spain, about 20 years ago, persimmon was grown for local consumption. The selection of cultivar 'Rojo Brillante' (PVA) having high fruit yield and quality and the application of the technique for removing astringency without losing fruit firmness led to the expansion of the persimmon culture in the 1990s [4]. Israel and Turkey that have lower production have significantly increased their production (respectively, 36,592 and 33,470 tonnes). On the other hands, while Japan's and Italy's production were 393,500 tonnes and 70,740 tonnes in 1961, they have dramatically decreased to 240,600 and 39,149 tonnes in 2014.

About 5% of the world's total persimmon production is exported as fresh fruit. The rest of persimmon is consumed in the internal market, and a good part of production is dried or processed. Although Azerbaijan is the sixth persimmon producer country, it is the first fresh fruit persimmon exporting country in the world. In 2013, Azerbaijan exported 95,118 tonnes of persimmon fruits. It is followed by Spain (40,121 tonnes), China mainland (35,799 tonnes), Israel (13,084 tonnes) and Poland (12,142 tonnes) (**Table 3**). Further, Russia (114,596 tonnes), Kazakhstan (58,464 tonnes) and Germany (30,233 tonnes) are the largest persimmon-importing countries in the World (**Table 4**).

Persimmons have proved to be highly adaptable to a wide range of climate conditions, ranging from subtropical coastal regions to mild coastal areas and warm inland temperate areas. Persimmons do best in areas that have moderate winters and relatively mild summers. Generally, astringent varieties prefer cooler climatic conditions than non-astringent varieties. Non-astringent cultivars require warmer growing conditions. If the cultivars of non-astringent types are grown in cooler regions, the fruit flesh may not lose all of its astringency and have lower sugar content at harvest. They can tolerate temperatures of  $-18^{\circ}\text{C}$  when fully dormant. Persimmons need only a short chilling period (about 100–400 h below  $7.2^{\circ}\text{C}$ ). The chilling requirement of non-astringent varieties is lower than that of astringent varieties. If the dormancy is broken or the chilling requirement of the variety has been supplied early by the warming climate, the new shoots can be damaged by a late spring frost. The leaves are killed by  $-3.3^{\circ}\text{C}$  when growing. However, persimmons generally bloom late in the spring

Year	China	Korea	Spain	Japan	Brazil	Azerbaijan	Taiwan	Uzbekistan	Italy	Israel	Turkey*	World
1961	495,000	13,271	—	393,500	15,298	—	2250	—	70,740	—	—	990,079
1962	440,000	16,594	—	322,500	16,005	—	2109	—	72,830	—	—	870,058
1963	475,000	14,114	—	383,500	16,239	—	1804	—	75,200	—	—	965,877
1964	465,000	23,602	—	464,000	17,198	—	2340	—	73,500	—	—	1,045,660
1965	515,000	23,510	—	346,400	19,988	—	1997	—	72,000	—	—	978,915
1966	495,000	22,075	—	419,300	19,586	—	2101	—	71,400	—	—	1,029,482
1967	475,000	23,609	—	504,400	20,037	—	2203	—	73,600	—	—	1,098,869
1968	470,000	34,579	—	450,100	20,427	—	2333	—	73,300	—	—	1,050,764
1969	414,000	33,854	—	444,100	20,849	—	2152	—	74,000	—	—	988,980
1970	455,000	30,310	—	342,700	21,659	—	2341	—	59,600	—	—	911,635
1971	427,000	22,887	—	303,200	21,558	—	2411	—	59,200	—	—	836,286
1972	480,000	31,115	—	306,900	25,188	—	2359	—	61,700	—	—	907,292
1973	535,000	32,284	—	347,200	19,374	—	2515	—	59,000	—	—	995,403
1974	538,000	41,928	—	283,600	20,446	—	2745	—	63,500	—	—	950,249
1975	528,000	20,890	—	274,700	22,114	—	2843	—	60,000	—	—	908,607
1976	530,000	16,946	—	264,100	22,364	—	3826	—	60,690	1700	—	899,686
1977	525,000	30,138	—	275,400	23,801	—	4422	—	62,100	2400	—	923,321
1978	570,000	29,984	—	287,000	36,340	—	4463	—	57,800	1900	—	987,547
1979	506,500	33,386	—	263,900	40,385	—	5267	—	58,500	2500	—	910,503
1980	560,400	31,837	—	265,200	39,958	—	6238	—	61,100	3400	—	968,198
1981	506,000	39,254	—	260,500	37,598	—	7313	—	62,800	5600	—	919,207

Year	China	Korea	Spain	Japan	Brazil	Azerbaijan	Taiwan	Uzbekistan	Italy	Israel	Turkey*	World
1982	482,000	57,807	—	333,700	38,396	—	6621	—	65,400	8100	—	992,416
1983	553,000	91,052	—	309,900	45,298	—	7850	—	76,700	12,700	—	1,096,858
1984	608,000	68,812	—	297,400	41,915	—	9525	—	78,400	13,900	—	1,118,477
1985	680,000	97,031	—	289,700	43,658	—	10,945	—	56,200	8100	—	1,186,165
1986	750,000	98,906	—	291,300	43,488	—	10,626	—	69,700	11,900	—	1,277,032
1987	820,000	75,677	—	290,200	45,000	—	11,811	—	69,820	19,200	—	1,332,767
1988	732,921	98,337	—	287,600	45,745	—	15,694	—	69,170	7000	—	1,257,684
1989	650,283	113,403	—	268,100	46,836	—	16,404	—	72,920	16,000	—	1,185,538
1990	624,773	95,758	0	285,700	46,712	—	15,457	—	68,770	17,200	—	1,157,241
1991	641,576	109,722	591	248,800	47,662	—	14,935	—	61,810	16,200	10,000	1,144,666
1992	724,329	155,111	591	307,700	46,611	—	14,636	—	66,546	17,390	10,000	1,336,383
1993	789,113	116,070	2963	241,900	48,086	—	16,380	—	56,753	15,780	10,000	1,290,971
1994	826,870	167,471	6895	302,200	55,406	—	15,100	—	48,999	13,800	9300	1,440,701
1995	969,363	194,585	10,826	254,100	51,685	—	16,440	—	61,300	11,000	9200	1,573,421
1996	1,025,219	210,766	14,757	240,500	52,534	—	15,811	—	67,800	18,200	9400	1,649,285
1997	1,075,417	239,570	18,688	301,700	52,198	—	19,018	—	59,800	16,800	10,000	1,787,234
1998	1,314,136	260,671	21,842	260,100	60,423	47,900	18,962	—	62,000	17,400	10,500	2,068,143
1999	1,481,327	273,846	29,469	286,000	64,096	60,300	25,754	—	40,769	14,000	11,500	2,280,753
2000	1,591,906	287,847	33,000	278,800	63,300	70,300	23,891	16,000	42,450	14,186	12,000	2,427,646
2001	1,584,660	270,338	34,500	281,800	105,000	86,000	26,169	16,000	48,240	14,900	13,500	2,473,149
2002	1,740,591	281,143	36,050	269,300	141,364	104,800	34,747	16,500	54,170	35,700	15,000	2,720,098

Year	China	Korea	Spain	Japan	Brazil	Azerbaijan	Taiwan	Uzbekistan	Italy	Israel	Turkey*	World
2003	1,795,110	249,207	45,200	265,000	158,131	114,899	38,247	17,000	47,000	40,100	15,000	2,776,510
2004	1,998,214	299,046	45,800	232,500	162,288	48,089	36,177	19,000	57,110	38,700	17,000	2,943,525
2005	2,185,041	363,822	51,950	285,900	164,849	108,965	27,111	21,000	51,332	48,000	18,000	3,314,731
2006	2,320,346	352,822	63,000	232,700	168,274	124,485	26,395	27,213	53,100	24,606	19,297	3,399,970
2007	2,574,143	395,614	67,000	244,800	159,851	128,407	32,962	28,000	52,500	37,347	23,713	3,727,432
2008	2,710,988	430,521	95,400	266,600	173,297	132,179	33,899	31,000	51,600	45,350	24,302	3,977,744
2009	2,834,165	416,705	100,200	258,000	171,555	135,549	37,032	40,000	51,593	32,291	25,281	4,084,050
2010	2,875,600	390,630	125,280	189,400	167,215	142,188	58,401	38,000	48,165	28,201	26,277	4,070,375
2011	3,187,239	390,820	159,400	207,500	154,625	146,084	90,100	53,400	50,347	29,271	28,295	4,476,946
2012	3,417,586	401,049	212,300	253,800	158,241	140,082	81,894	56,000	51,165	31,292	32,392	4,813,100
2013	3,538,823	351,990	242,800	214,700	173,169	143,106	63,694	75,000	41,858	35,692	33,232	4,890,000
2014	3,730,800	428,363	245,000	240,600	182,290	140,405	72,674	66,000	39,149	36,592	33,470	5,190,624

Source: FAO, 2017. \* Represents data from Turkish Statistical Institute (TUIK), 2017.

**Table 1.** World persimmon production amount (tonnes).

Rank	Country	Production in tonnes	Area (ha)	Yield (kg/ha)
1	China, mainland	3,730,800	931,907	4003.4
2	Republic of Korea	428,363	27,988	15,305.2
3	Spain	245,000	13,370	18,324.6
4	Japan	240,600	21,300	11,295.8
5	Brazil	182,290	8323	21,902.0
6	Azerbaijan	140,405	8712	16,116.3
7	China, Taiwan Province of	72,764	5263	13,825.6
8	Uzbekistan	66,000	4218	15,647.0
9	Italy	39,149	2531	15,467.8
10	Israel	36,592	1374	26,631.7
11	Turkey*	33,470	2062	16,232.6
12	New Zealand	2600	164	15,853.7
13	Iran (Islamic Republic of)	2452	275	8926.6
14	Nepal	1918	288	6667.4
15	Australia	715	86	8272.1
16	Slovenia	801	70	11,442.9
17	Mexico	175	18	9722.2
18	Chile	—	102	—
<b>World total production</b>		<b>5,190,624</b>	<b>1,028,051</b>	<b>5049.5</b>

FAO stat [2]. <http://www.faostat.com>. \*TÜİK [3]. Turkish Statistic Council records.

**Table 2.** Persimmon production amount (ton), planted area (ha) and yield (kg ha<sup>-1</sup>) in 2014.

(mid-April) to avoid spring frosts. On the other hand, persimmon has been damaged by early-autumn frosts. Early-autumn frosts can lead to skin blemishes on fruit and early defoliation. Persimmon does not tolerate wind. It does not provide a good fruit yield and quality, if strong winds occur during the growing season. Fruit is also prone to wind rub from leaves and branches causing skin blemish on fruit. Windbreakers can be used to reduce the wind speed.

Full sun with some air movement is recommended for persimmon trees in inland areas, although they will tolerate some partial shade. But trees do not produce well in the high summer heat of desert regions, which sunburn the bark.

Kaki persimmons are drought tolerant. Persimmon trees can withstand drought, but fruit yield and quality (especially size) are reduced. Also, adequate moisture in the soil is required to produce sufficient shoot growth and formation of flower buds for next year's crop. The trees should be irrigated during dry periods.

The persimmons trees can grow well on a wide range of soil types but do best in deep, well-drained loam soils with a good supply of organic matter. Heavy clay loam soils that are prone

Rank	Country	Amount (tonnes)
1	Azerbaijan	95,118
2	Spain	40,121
3	China, mainland	35,799
4	Israel	13,084
5	Poland	12,142
6	Lithuania	9057
7	Republic of Korea	7379
8	Netherlands	6957
9	Georgia	6781
10	South Africa	5809
	<b>World total</b>	<b>232,247</b>

**Table 3.** Top 10 countries with highest persimmon exports in 2013.

Rank	Country	Amount (tonnes)
1	Russian Federation	114,596
2	Kazakhstan	58,464
3	Germany	30,233
4	Belarus	14,788
5	France	12,929
6	Italy	11,427
7	Lithuania	10,177
8	Poland	9715
9	Thailand	6997
10	Canada	6407
	<b>World total</b>	<b>323,858</b>

**Table 4.** Top 10 countries with highest persimmon imports in 2013.

to water-logging should be avoided. The preferred soil pH for optimum tree growth is in the range of 6.0–7.5. However, persimmon trees can tolerate a wider variety of conditions than most fruit trees.

Pest and disease problems: protection of fruits from bats and birds are required. Fruit flies are the potential problem as are aphids and mealybugs. Persimmon trees are also susceptible to collar rot, thus keeping mulch clear of the trunk is required.



## 2. Origin and history

Zeven and Zhukovsky [5] suggested that persimmon (*D. kaki*) has a primary center of genetic origin in the mountains of central China and a secondary center in Japan. Persimmon cultivation in China began more than 2000 years ago, and it is also scientifically known as *D. chinensis*. In China, it is found wild at altitudes up to 6000–8000 ft. [6]. It spread from China to Korea and to Japan many years ago. Since from prehistoric times, persimmon is consumed as food source in these countries. There are some trees that are 400–500 years old. It was imported in Europe (South France) for the first time in 1760. Thereafter it spread to the Mediterranean coast (Italy, Spain, Greece, Turkey and Algeria). The persimmon plant was introduced in North America (California, Florida), South America (Brazil) and Australia in the mid-1800s. Early in the fourteenth century, the explorer Marco Polo recorded the Chinese trade in persimmons [7]. Its cultivation has recent traditions in western countries where it is present only since the second half of the nineteenth century. Currently, persimmon is one of the most important fruit crops in Asian countries and, there is also steady increase in its production in some European countries.

## 3. Botany of persimmon

The genus *Diospyros* contains hundreds of plant species and are distributed in the tropical and subtropical regions of the world. Four species of them have commercial importance. *D. kaki* L. is the most widely cultivated species of the *Diospyros* genus. *D. kaki* is also known as the persimmon, Japanese persimmon, Oriental persimmon, Japanese persimmon, Kaki, Asian persimmon. It has been reported that wild type *D. kaki* exists in the forests of China [8, 9]. The other species are *D. lotus* L. (the date plum), *D. virginiana* L. (native American persimmon) and *D. oleifera* Cheng [10].

The origin of *D. kaki* and its relationship to other *Diospyros* species is not well understood. The persimmon culture was known to occur in the fifth or sixth century in China [9]. In addition to *D. kaki*, *D. lotus* and *D. oleifera* also have been cultivated as fruit crop. *D. lotus* has been consumed as a fresh as well as dried fruit, and it is a source for tannin [9, 10]. Another important species known as a fruit crop is *D. virginiana*, of the eastern United States origin. This species which is consumed as fresh and processed is grown on a much smaller scale and is not yet considered a commercial crop [11]. These species are quite important as horticultural crops among the *Diospyros* species of temperate origin. On the other hands, *D. rhombifolia* originated from China is an ornamental plant which bears tiny attractive-colored fruit on a dwarf tree [9]. There are other species such as *D. digyna* (black sapote), *D. discolor* and *D. decandra* that have originated in the tropics and subtropics and produce edible fruits.

In the genus *Diospyros*, there are species and varieties having diploid ( $2n = 2x = 30$ ), tetraploid ( $2n = 4x = 60$ ), hexaploid ( $2n = 6x = 90$ ), nonaploid ( $2n = 9x = 135$ ) and dodecaploid ( $2n = 12x = 180$ ) chromosome number. Therefore, it is thought that the basic chromosome number of the genus *Diospyros* is 15 [9, 12]. The chromosome numbers of some wild species of genus *Diospyros*

(*D. oleifera*, *D. glandulosa*, *D. confertiflora*, *D. discolorare*, *D. ehretioides*, *D. lycioides*, *D. mollis*, *D. rhodocalyx* and *D. sumatrana*) are  $2n = 30$ , except for  $2n = 60$  for *D. rhombifolia* and  $2n = 90$  for *D. ebenum* [13–15]. *D. kaki* L. is a hexaploid ( $2n = 6x = 90$ ). However, octoploid ( $2n = 8x = 120$ ) cultivars such as Hasshu and nonaploid ( $2n = 9x = 135$ ) cultivars such as ‘Hiratanenashi’ and ‘Tonewase’ have also been reported [14–16]. On the other hand, *D. virginiana* has two karyotypes with  $2n = 60$  and  $90$  [15, 17], while *D. lotus* is diploid ( $2n = 2x = 30$ ) [14].

#### 4. Pomological classification

Persimmon fruit is highly astringent due to soluble tannins in the vacuoles of the fruit flesh. However, some cultivars lose astringency naturally on the tree as fruit ripens, while others retain astringency until maturity. Therefore, persimmons are classified into two major groups (based on the presence or absence of astringency in the fruit at maturity) as astringent (A) and non-astringent or sweet (NA) cultivars. Water-soluble tannins which cause astringency in the flesh of astringent types decrease as the fruit softens and becomes edible. However, astringency can be removed by various chemical treatments. Carbon dioxide gas or alcohol can be used to remove astringency, while the fruit remains firm. If ethylene is used for removing astringency, the fruit softens very quickly. Fruit of the non-astringent types naturally loses astringency, while the fruit is still firm. Thus, the fruit of non-astringent types is edible either the flesh is firm or soft [10, 12, 18].

Each group can be further subdivided, based on their response to pollination [18]. The amount of dark flesh coloration around the seeds varies in cultivars and changes in flesh color are related to seed formation, not pollination. In pollination variant types (PV), the flesh is dark and streaked around the seeds, but clear orange when seedless. When pollination is poor and only one or several seeds are formed, a dark area develops around the seeds, but the remaining flesh is light colored. The pollination variant types include cultivars that are astringent when they have several seeds or seedless (PVA), as well as partially or totally non-astringent when they have only one or a few seeds (PVNA). Also, in astringent cultivars of the pollination variant type, fruit which has a great degree of the dark flesh is non-astringent even when the fruit flesh is firm [10, 18].

Pollination constant (PC) types lack the dark streaking regardless of seed formation. The flesh color of pollination constant astringent (PCA) cultivars is not influenced by pollination and it does not develop dark flesh around the seeds. Pollination constant non-astringent (PCNA) persimmons are always edible when still firm, regardless of whether or not pollination has occurred.

PVA types can vary to either PCA or PVNA depending on several situations. If PVA type does not have any seed for some reason or when PVA persimmon varieties are cultivated without pollinators, the fruit has clear orange flesh and remains astringent (PCA) such as the Spanish variety ‘Rojo Brillante’ and Japanese variety ‘Hiratanenashi’. Similarly, when PVA type has enough seeds (usually four or five) after pollination, the fruit has a great degree of dark flesh and loses astringency (PVNA) such as ‘Nishimura Wase’.

## 5. Commercial and recently improved persimmon varieties

The fruits of two species (*D. kaki* L. and *D. virginiana*) in the genus *Diospyros* have commercial importance. In China, using native persimmon germplasm, several common persimmon varieties were developed. However, they all are PCA with the exception of 'Luo Tian Shi' [19, 20]. Persimmon is the main species cultured for edible fruit production in northern China. Recently, 'Jirou', 'Youhou', 'Taishuu' and 'Fuyu' among PCNA cultivars are gaining popularity. Persimmon growing regions are also spreading widely in Japan and Korea, thus some old well-known Persimmon varieties which were still being produced were selected from these countries. Persimmon has been a major fruit crop in Japan for many years [21] and for Japanese persimmon commercial production, 'Fuyu' (PCNA), 'Hiratanenashi' (PVA) and 'Tonewase' have been the three important cultivars. About 57% of the total area is devoted to these varieties [22]. Other varieties growing in Japan are 'Kosyu Hyakume', 'Matsumotowase Fuyu', 'Early ripening Jiro', 'Ichidagaki', 'Jiro Dojohachiya' and 'Taishu'. However, newly released cultivars such as 'Reigyoku' and 'Taiho' are also available. In Korea, non-astringent varieties have increased, while astringent varieties have decreased. In the recent years, amount of production of non-astringent varieties are higher than those of astringent varieties. Major cultivar of non-astringent type is 'Fuyu', which accounts for almost 82% in total production of persimmon, and 'Jiro' with 9.8% [23].

In Taiwan, 'Suzhou', 'Niouhsin' and 'Shihshih' local PCA are the major commercial varieties used. 'Fuyu' and 'Jiro' are the main PCNA varieties [24]. Countries such as Azerbaijan and Uzbekistan focused on local astringent cultivars. In Brazil, the most cultivated persimmon cultivars include 'Rama Forte' and 'Giombo', which belong to the PVA group, 'Taubate' which is continually astringent with yellow flesh either with or without seeds (PCA), and 'Fuyu', which belongs to the PCNA group [25, 26].

'Fuyu', 'Hana Fuyu' and 'Ichikikei Jiro' cultivars which are PCNA and 'Hachiya' (PCA) are commonly produced in the USA [10, 18]. In new persimmon growing countries such as New Zealand and Australia, most of the cultivation area is devoted to 'Fuyu' [27]. In Spain, the most produced cultivars are 'Rojo Brillante' (Figure 1) and 'Triumph' which can be stored for



**Figure 1.** Fruits and tree of 'Rojo Brillante' cultivar.





**Figure 2.** Fruits and tree of 'Triumph' cultivar.

a long time [28]. In Italy, almost 90% of the persimmon production is Kaki Tipo (PVNA), the rest of production is other PVNA varieties (Vainiglia, Mercatelli and Moro) and PCNA cultivars such as Hana Fuyu, Jiro and Gosho [29].

Israel has its own cultivar, Triumph (**Figure 2**) which is sold under the name of Sharon fruit, and it is planted on 95% of the total area devoted to persimmon [27]. Also, persimmon production in South Africa is based on Triumph [30]. In Turkey, a great amount of production is PCA and PVNA varieties, which are selected from Turkey. However, recently introduced PCNA cultivars such as Fuyu, Hana Fuyu, Jiro and Izu have become popular with the growers. The new orchards with 'Fuyu' and 'Hachiya' cultivars have been established.

## 6. Persimmon germplasm resources

Persimmon originated from China, but it has been cultivated and produced mostly in Japan [31]. Persimmon has limited amount of production in the rest of the World. However, Spain, Italy, Israel and Brazil are now producing important amounts and these countries have developed their own cultivars such as 'Rojo Brillante' in Spain, 'Kaki Tipo' in Italy, 'Triumph' in Israel and 'Lama Forte' in Brazil. Recently, Australia and New Zealand have started to produce persimmon mainly for export, and the USA is also producing persimmon on a small scale.

Greene and Morris [32] indicated that germplasm collections are a source of genetic diversity to support crop improvement and botanical research as well as to support conservation efforts [33]. For the specific breeding objectives, these variations can either be created spontaneously or artificially by budwood mutations or cross breeding. The importance of germplasm can be explained by the variation of plant material. Therefore, recording and registration of genetic resources is critical for breeders in terms of improving new varieties.

Currently, more than 950 cultivars of persimmon exist from the subtropical to temperate regions of China [34]. There is only 1 genus and 63 species in persimmon family and most of them are distributed in tropic and sub-tropic regions of Hainan, Yunnan, Guangdong, Guangxi and Fujian provinces in China. The 63 species originated from this genus in China. Among these species *D. kaki* Thunb., *D. oleifera* Cheng., *D. lobata* L., *D. discolor* Willd., *D. pottingensis* Merr. et Chun., *D. lotus* Linn., *D. glaucifolia* Metc., *D. rhombifolia* Hemsl. and *D. morrisiana* Hance. have been cultivated as fruit crops [35]. There are 550 accessions including most cultivars native to China and some native to Japan and Korea.

Aside from this exceptional existence of a PCNA type cultivar in China, almost all non-astringent type cultivars were developed in Japan. Historical records show that 'Zenjimarū', known to be the oldest PVNA type cultivar, was found in the beginning of the twentieth century, and that 'Goshō', was the first PCNA type cultivar, which was recorded in the seventeenth century [36]. In the beginning of the nineteenth century, 'Fuyu' and 'Jiro' were recorded as the most popular PCNA type cultivars. According to a nationwide survey on persimmon cultivars in Japan (Agricultural Research Station 1912), there were only 6 PCNA type cultivars in contrast to 401 PVNA type cultivars among more than 1000 cultivars collected from all over Japan. This means that in addition to its more recent appearance, the PCNA type probably has very narrow genetic variability. A total of 40 PCNA cultivars, including bud sports, which may cover almost all PCNA type cultivars currently existing in Japan, are now preserved at the National Institute of the Fruit Tree Science (NIFTS) in Akitsu, Hiroshima [9]. There are many astringent and PVNA local cultivars throughout Japan. The current conservation in Japan consists of approx. 600 genotypes [37].

In Korea, 233 local cultivars were collected at the branch of Experimental Station at Kim-hae during 1959–1969, and 74 superior cultivars were selected for persimmon cultivation after identifying the name of 188 cultivars among these local cultivars. In Korea, interest in persimmon cultivation is increasing and two experimental stations for persimmon have been established, the one for non-astringent persimmon was established in 1994 and the other for astringent persimmon established in 1995. In addition, a breeding program for obtaining new PCNA cultivars was started in 1995 by crosses among PCNA cultivars that were introduced from Japan. The breeding objectives in Korea are focused on obtaining superior PCNA cultivars with good eating qualities, large fruit and early ripening characteristics [9].

In Europe, persimmon is considered a secondary fruit tree species; only few countries, located in the Mediterranean area, are interested in a large-scale production.

Persimmon was introduced in Italy at the end of the nineteenth century. Later in Tuscany, the interest for this new species was increased and the genotypes were collected together with exotic and local varieties of fruit tree species (citrus, peaches and plums among others). As early as 1940, the University of Florence collected 11 accessions from the USA and France, or as local varieties and characterized them. The persimmon collection of Florence consisted of 52 cultivars and was totally destroyed by winter frost in 1985. Then a new germplasm orchard was established by introducing new accessions from Japan. A French germplasm was recorded at the beginning of the twentieth century. The Spanish collection was created in 1993 with material from Italy (54.2% of accessions) and from Spanish institutions and nurseries (45.8%) [38].

Persimmon was introduced into Brazil's São Paulo state in 1890. However, its cultivation expanded around 1920 with Japanese immigration. São Paulo is the main persimmon producing state. Rio Grande do Sul state has the second largest persimmon production of Brazil. In recent years, the persimmon acreage has increased and the trend is to continue crop expansion. 'Fuyu', 'Rama Forte', 'Giombo' and 'Taubaté' are the cultivars grown in Brazil. In Azerbaijan, persimmon production is widely spread since 1998, although its history has deep roots [2]. In terms of persimmon genetic resources in Israel, only high commercial value cultivars are collected.

Although the exact date of the introduction of persimmon to Anatolia is unknown, it is clear that it dates back to rather old times [39, 40]. Persimmon was introduced to Turkey from Russia via the Black Sea region. Turkey has main persimmon species (*D. kaki*, *D. lotus* and *D. oleifera*). *D. oleifera* can be seen only in the Mediterranean region of Turkey, while *D. lotus* grows as wild in Northern Anatolia and is used as dried fruits in this region. *D. kaki* and *D. oleifera* have been introduced from other countries at least 200 years ago. During this time, continuous propagation of persimmon by its seeds resulted in genetic diversity in *D. kaki* trees due to the high heterozygosity. Therefore, in the northeastern part of Turkey, persimmon trees differ from another in terms of fruit productivity, yield, shape, size, astringency and plant growth. This diversity in persimmon population in Turkey provided a great opportunity to the breeders for selection programs. As a result, the breeders were able to identify many promising clones in different parts of Turkey. A germplasm collection in the Black Sea region in Turkey with selected promising genotypes has been established.

First studies on persimmon in Turkey were started to introduce the foreign cultivars by the Ministry of Agriculture in 1967. Then, some selection studies were done in different parts of Turkey. After 1989, the total number of the known cultivars and types reached up to 74. Most of these varieties were introduced from Italy and some of them were from Israel, Japan, France and Pakistan especially after the attempts made by the Cukurova University, in Eastern Mediterranean, Turkey.

Selection of different genotypes was started by the Department of Horticulture of Cukurova University, by Department of Horticulture of Ondokuzmayıs University in Black Sea and by Citrus Research Institute of Antalya belonging to the Ministry of Agriculture in Western Mediterranean regions. Recently, the wider selections have been carried out especially on Black Sea coast by Citrus Research Institute of Antalya for 1 year and they presently have 43 promising candidate clones. Most even totality of the selections is astringent type. It seems to be rather difficult to find non-astringent types in Turkey. Yilmaz et al. [41] established a characterization study on persimmon genetic resources collected from Turkey. These germplasms were preserved with commercial cultivars in an *ex situ* germplasm preservation orchard located at the Cukurova University, Turkey. Persimmon genotypes were characterized based on their morphological traits. The collection comprising traditional genotypes, local accessions and also global varieties were collected from five different provinces of the Mediterranean region of Turkey where persimmon is widely produced. A total of 48 persimmon genotypes and cultivars were morphologically characterized, using 59 morphological and agronomic traits (Table 5).

No	Cultivar and selections	Scientific Name	Origin	Type of astringency
1	Shakoku	<i>Diospyros kaki</i> L.	France	PCA
2	<i>Diospyros lotus</i>	<i>Diospyros lotus</i>	France	PCA
3	<i>Diospyros virginiana</i>	<i>Diospyros virginiana</i>	Israel	PCA
4	Seedless Mardan	<i>Diospyros kaki</i> L.	Pakistan	PCA
5	Yesil Hurma	<i>Diospyros oleifera</i>	Selection from Adana-ME-Turkey	PCA
6	07 TH 13	<i>Diospyros kaki</i> L.	Selection from Antalya-ME-Turkey	PCA
7	07 TH 14	<i>Diospyros kaki</i> L.	Selection from Antalya-ME-Turkey	PVA
8	07 TH 17	<i>Diospyros kaki</i> L.	Selection from Antalya-ME-Turkey	PVA
9	07 TH 18	<i>Diospyros kaki</i> L.	Selection from Antalya-ME-Turkey	PVA
10	31 TH 01	<i>Diospyros kaki</i> L.	Selection from Hatay-ME-Turkey	PVA
11	31 TH 03	<i>Diospyros kaki</i> L.	Selection from Hatay-ME-Turkey	PCA
12	55 TH 05	<i>Diospyros kaki</i> L.	Selection from Samsun-BS-Turkey	PVA
13	Fatsa-1	<i>Diospyros kaki</i> L.	Selection from Ordu-BS-Turkey	PVA
14	Sarı Yenen	<i>Diospyros kaki</i> L.	Selection from Istanbul-MR-Turkey	PCA
15	Cekirdekli	<i>Diospyros kaki</i> L.	Selection from Adana-ME-Turkey	PVA
16	Saijo	<i>Diospyros kaki</i> L.	Israel	PCA
17	Hachiya	<i>Diospyros kaki</i> L.	Italy	PCA
18	Guilbecky	<i>Diospyros kaki</i> L.	Italy	PCA
19	BST-29	<i>Diospyros kaki</i> L.	Italy	PCA
20	Fennio	<i>Diospyros kaki</i> L.	Italy	PCA
21	Lycopersicon	<i>Diospyros kaki</i> L.	Italy	PCA
22	Farmacista Honorati	<i>Diospyros kaki</i> L.	Italy	PCA
23	Fujiwara O'Gosho	<i>Diospyros kaki</i> L.	USA	PCNA
24	Triumph	<i>Diospyros kaki</i> L.	Israel	PCA
25	Vainiglia	<i>Diospyros kaki</i> L.	Pakistan	PVNA
26	Aman Kaki-1	<i>Diospyros kaki</i> L.	Pakistan	PVNA
27	Sirin Hurma	<i>Diospyros kaki</i> L.	Iran	PVA
28	Nishimura wase	<i>Diospyros kaki</i> L.	Italy	PVA
29	Mikatani O'Gosho	<i>Diospyros kaki</i> L.	Italy	PVNA
30	Mandarino	<i>Diospyros kaki</i> L.	Italy	PVNA
31	Bruniquel	<i>Diospyros kaki</i> L.	Italy	PVNA
32	Aman Kaki-2	<i>Diospyros kaki</i> L.	Italy	PVNA
33	Koshu Hyakume	<i>Diospyros kaki</i> L.	Japan	PVA
34	Mizushima O'Gosho	<i>Diospyros kaki</i> L.	Italy	PVNA
35	Chienting	<i>Diospyros kaki</i> L.	USA	PVA



No	Cultivar and selections	Scientific Name	Origin	Type of astringency
36	Jiro C – 24,276	<i>Diospyros kaki</i> L.	Italy	PCNA
37	Kawabata O’Gosho	<i>Diospyros kaki</i> L.	Italy	PCNA
38	Giant Fuyu	<i>Diospyros kaki</i> L.	Israel	PCNA
39	Tipo Kaki	<i>Diospyros kaki</i> L.	Pakistan	PVNA
40	Shogatsu	<i>Diospyros kaki</i> L.	Italy	PVNA
41	Giboshi	<i>Diospyros kaki</i> L.	Italy	PVNA
42	Thiene	<i>Diospyros kaki</i> L.	Italy	PVNA
43	Moro	<i>Diospyros kaki</i> L.	Italy	PVNA
44	Brazzale	<i>Diospyros kaki</i> L.	Italy	PVNA
45	Kirakaki	<i>Diospyros kaki</i> L.	Italy	PVNA
46	Akouman Kaki	<i>Diospyros kaki</i> L.	Italy	PVNA
47	Kurokuma	<i>Diospyros kaki</i> L.	Italy	PVNA
48	Hyakume	<i>Diospyros kaki</i> L.	Italy	PVNA

Table 5. Origins of persimmon accessions.

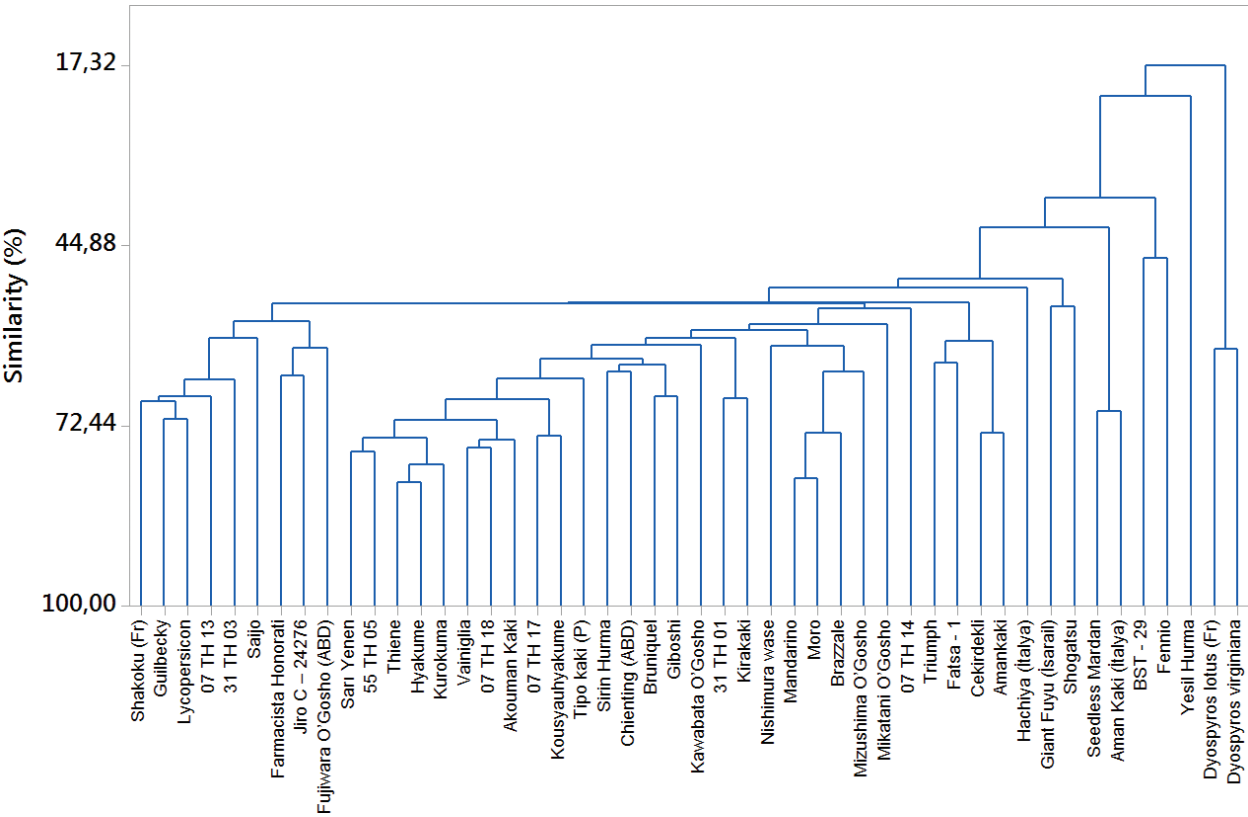


Figure 3. Dendrogram of persimmon accessions collected from Turkey obtained from cluster analysis of 59 agro-morphological traits using average method.



From them, 9 traits were related with plant growth, 5 with leaves, 7 with flowers, 32 with fruits and 6 with seeds. As a result of the morphological characterization, persimmon varieties and types were classified by PCA, PVA, PCNA and PVNA. Besides, data obtained by characterization were subjected to similarity coefficient, principal components and cluster analyses to study phenotypic relationships among these genotypes. As a result of their study, the 12 factor scores represented 74.75% of the total multivariate variation, and cluster analysis indicated that the similarity index of the population consisting of the investigated genotypes ranged between 81.09 and 17.32% (**Figure 3**).

## 7. Persimmon breeding and genetic improvement

Breeding aims for persimmon emphasized on enhanced fruit quality such as fruit weight, shape, color, soluble solids content, fruit cracking, fruit ripening time, high productivity, long shelf life, parthenocarpy and sex expression. Selection breeding is the most common breeding technique in persimmon due to the fact that persimmon breeding is mainly hindered by its high ploidy level and by its complex sex expression [42]. Because somatic and bud sport mutations affect the fruit traits, new lines of persimmon are frequently improved by bud mutations [43, 44]. Also, there are many seed propagated populations in persimmon growing countries of the world especially in native Asian countries. Many established cultivars are chance seedlings selected by growers or researchers in Japan, China and Korea. There are also many selections from introduced genotypes or seedling populations in the USA, Israel, India, Australia, New Zealand, Taiwan, Malaysia and some other countries. 'Kaki Tipo' in Italy, 'Lama Forte' in Brazil, 'Triumph' in Israel and 'Rojo Brillante' in Spain are cultivars that developed from bud sports [45]. 'Fuyu', 'Hachiya', 'Hiratanenashi', 'Izu', 'Jiro' and 'Saijo' cultivars were selected from shoot of bud sports in Japan and these selected cultivars are extensively growing all over the world. Early ripening bud sports of 'Fuyu' (Matsumotowase-Fuyu) and 'Jiro' (Maekawa-Jiro) were also found in a farmer's orchard. Early ripening Matsumotowase-Fuyu showed fruit cracking tendency as 'Fuyu'. Recently, a small fruit mutant, 'Totsutanenashi' (TTN), was discovered in Japan as a bud sport mutant of the leading cultivar Hiratanenashi (HTN) [44]. Following cultivars were also obtained by bud sports in Japan: 'Uenishiwase' (PCNA) and 'Kyi-joh' (PCNA) are bud mutation of 'Matsumotowase-Fuyu'; 'Sunami' (PCNA) and 'Tanbawase-Fuyu' (PCNA) are bud mutation of 'Fuyu'; 'Aisyuhou' (PCNA) is bud mutation of 'Maekawa-Jiro'; 'Tonewase' (PVA), 'Ohtanenashi' (PVA) and 'Kohshimaru' (PVA) are bud mutation of 'Hiratanenashi' [42]. 'Nantongxiaofangshi' variety having dwarfness character is a persimmon that has been found in Nantong. 'Nantong small persimmon' (*D. kaki* Linn. cv. Nantongxiaofangshi) is a rare and dwarf variety of persimmon found in 1982 in Jiangsu Province Nantong City Fruit resource survey.

The height of the adult tree is only about 2 m, which is approximately equal to 60% that of the standard type growing under the same conditions [46]. 'Hasshu' persimmon (*D. kaki* Thunb.) is a dwarf cultivar originated by a bud sport from the leading persimmon cultivar 'Hiratanenashi' in Hiroshima prefecture, Japan in 2005. Its somatic polyploidy ( $2n = 120 = 8x$ ) was confirmed by flow cytometric analysis and chromosome observation. Although non-aploid 'Hiratanenashi' and some of its bud sports are known to be seedless, 'Hasshu' produces regular seeds with the ability to germinate [16].

Main objective of persimmon breeding has been to produce commercially attractive cultivars of the PCNA type which can be eaten without any postharvest treatment [46]. Therefore, PCNA fruit are the most desirable for fresh consumption because it is not necessary to apply any postharvest treatment in order to remove the astringency. Hence, the breeding of new PCNA cultivars is the most popular objective in the entire persimmon growing countries.

Although persimmon is produced in Brazil, Israel, Italy, Spain, Azerbaijan, Uzbekistan, New Zealand and Australia, new persimmon cultivars developed by cross breeding have been released only in Japan and Korea [21] and also in these countries persimmon cultivars have been selected over time for commercial production. Hybridization method can also be used in persimmon breeding. In Japan, hybridization method has been used for fruit ripening time, crack-resistance and large fruit size in persimmon.

Persimmon breeding is complex, and results are not always as expected, especially when working on the PCNA group [42]. Researchers found that fruit ripening time is under additive and quantitative control. The tendencies of persimmon fruit are quantitatively inherited traits, the non-cracking cultivars are homozygous, whereas cultivars with cracking are heterozygous. Also, it has been claimed that fruit weight is a quantitative characteristic with high broad-sense heritability [21]. Many crosses performed using large and small fruit size parents indicated that small fruit alleles were dominant to large size alleles [48]. Complete loss of astringency is important for commercial persimmon production. Generally, a little astringency remains in PCNA fruit at maturity in cooler regions, so that they are commercially produced in warm regions. Incomplete loss of astringency results from not only environmental factors but also genetic factors [21]. According to the criteria established for persimmon cultivars, persimmon can be categorized into two major groups, PCNA type consisting of two subcategories, Chinese PCNA (CPCNA) and Japanese PCNA (J-PCNA). The second group is non-PCNA type consisting of three subcategories: PCA, PVNA and PVA [49]. Japanese PCNA cultivars are based on a recessive character and their genetic resources are very few. Repeated crossings within the narrow gene pool cause inbreeding depression, which hinders tree vigor, fruit yield and size. Therefore, studies have been ongoing to obtain new cultivars through the backcross (PCNA  $\times$  non-PCNA)  $\times$  PCNA since 1990. In 2007, 'Taiten' and 'Taigetsu', which are PVA cultivars, were derived from the cross of 'Kurokuma' (a local PVNA cultivar in Japan)  $\times$  PCNA cultivar 'Taishu'. Parthenocarpy in 'Taigetsu' is high [19]. The trait of natural astringency loss is dominant and controlled by the single locus *CPCNA* in Chinese PCNA persimmon [50]. At the end of the hybridization studies in Japan, PCNA cultivars 'Shinshuu', 'Soshu', 'Kanshu' and 'Kishu' were released as early ripening cultivars while 'Suruga', 'Youhou', 'Taishuu' and 'Yubeni' were released as medium to late ripening cultivars [21]. Other persimmon hybrids are 'Fuyuhana' and 'Ito'. 'Fuyuhana' were developed from a 'Fuyu'  $\times$  'Hanogoshu' cross as an alternative to 'Fuyu' and 'Jiro'. Ito is another hybrid obtained by crossing 'Fuyu'  $\times$  'Oku-Ogoshu' [51].

In persimmon, different genus can pollinate each other. Native American persimmon (*D. virginiana*) and Japanese persimmon (*D. kaki*) hybridization would set a goal of stabilizing and improving the variable flavors and cold hardiness of the native American persimmon. *D. kaki* and *D. virginiana* are apparently cross-incompatible; however a hybrid 'Rossiyanka' has been developed through embryo culture technique [51]. Rossiyanka is cold hardy, nearly seedless and it is smooth textured with Asian persimmon flavor [52]. Nikita's gift hybrid persimmon is unique hybrid of Asian and American persimmon; the fruit is sweet and flavorful [53].

In persimmon breeding programs, mutation breeding technique has also used. The main objectives of persimmon mutation breeding were focused on obtaining new cultivars with the positive agronomic features but with more diversity in ripening date, astringency and fruit characteristics from the PCNA types. However, obtaining PCNA type varieties is difficult due to the dominant inheritance of astringency, the limited number of cultivars which bear male flowers and the hexaploid inheritance of basic persimmon cultivars. Therefore, the PCNA type cultivars have low genetic diversity and crossing among these generally result in negative effects of inbreeding. Mutation breeding has been used as an alternative method for generating diversity in persimmon [54]. Some researchers studied to determine which gamma ray doses can be used in persimmon. Ray [51] claimed that 5–10 kR gamma doses obtained widest range of viability on cuttings, seeds and pollen of persimmon. In Spain, shoot buds of the persimmon ‘Rojo Brillante’ were subjected to various doses of gamma rays, 15 and 20 gray from a  $^{60}\text{Co}$  source. In this study, Naval et al. [55] found that the most favorable gamma irradiation dose combining survival and mutation induction was 20 gray. Two new varieties with similar fruit quality to ‘Rojo Brillante’, that allow to enlarge the persimmon harvest season in Spain, were selected [56].

## 8. Biotechnology and genomics

Biotechnology refers to the use of living organisms or their components to provide useful products in its broadest sense. Using biotechnology in plant breeding has become the most attractive method due to increasing knowledge in plant biotechnology and genomics. Improvements in the field of genomics have resulted in the development of huge quantities of useful new knowledge that greatly assists scientific plant breeding. Also, improvements in biotechnological techniques like plant tissue culture provided new methods for rapid production of high-quality, disease-free and true to type planting material.

In persimmon, biotechnological advances and molecular biology have been used for the classification of *Diospyros* species, *in vitro* propagation, regeneration from callus, root, protoplast and endosperm, ploidy manipulations, agrobacterium-mediated genetic transformation and marker-assisted selection. Molecular markers have been widely used for investigating the genetic relationships among persimmon genotypes. Akbulut et al. [57] compared persimmon genotypes by using random amplified polymorphic DNA (RAPD) and fatty acid methyl esters (FAME) data. The results showed that RAPD analyses could differentiate the relationship of persimmon (*D. kaki* Thunb.) genotypes used in their study. The authors suggested that more cultivars were needed as plant materials in terms of determining the degree of relationships of RAPD and FAME data which could help delimiting taxonomic classes within persimmon. Raddová et al. [58] indicated that RAPD and inter-primer binding site (i-PBS) were reliable enough to detect differences between the genetically close cultivars of persimmon. In addition, Badenes et al. [45] studied the genetic diversity of introduced and local Spanish persimmon cultivars as revealed by RAPD markers. The authors suggested that a correct identification of germplasm material from persimmon collections should be the first step in projects related to breeding or management of cultivar aimed at improving the crop. They also indicated that RAPD technology is adequate for fingerprinting persimmon. Yonemori et al. [59] studied the relationship between the European persimmon (*D. kaki* Thunb.) cultivars and Asian cultivars using AFLPs. The authors indicated

that the placement of several Japanese cultivars within the European cultivar group suggests that European cultivars were developed from Japanese germplasm relatively recent and differences among cultivars are much greater than differences among cultivar groups regarding AFLP markers. In addition, Guo and Luo [60] indicated that SSR markers are a valuable tool for the estimation of genetic diversity and divergence in *Diospyros*.

The main *in vitro* tissue culture techniques developed for persimmon deal with direct regeneration (from dormant buds and root tips) and indirect regeneration through callus from dormant buds, apices and leaves. Kochanová et al. [61] indicated that in the genus *Diospyros* L., biotechnological researches focused on quality improvement and preservation of the cultivars that has been economically cultivated. The authors also remarked that the genetic variability had been lost as only those limited cultivars that are popular among growers are grown. In recent years, studies were conducted on *in vitro* micro-propagation of persimmon [62], especially on Jiro [63] and Rojo Brillante [64]. Choi et al. [65] recorded an efficient and simple plant regeneration via organogenesis from leaf segment cultures of persimmon (*D. kaki* Thunb.). The authors indicated that the frequencies of adventitious shoot regeneration by 'Nishimurawase' and 'Fuyu' reached up to 100% and the regenerated shoots rooted successfully with over 80% efficiency. Yokoyama et al. [66] suggested that the meristematic nodule is a promising material for propagation and long-term conservation of 'Fuyu' variety. Naval et al. [67] recorded a protocol for plant regeneration of *D. kaki* Thunb. cv. 'Rojo Brillante' via organogenesis from leaf explants by using combined phytohormones and dosages. In addition, Naval et al. [67] studied somaclonal variation of 'Rojo Brillante' as a breeding tool by using various combinations of cytokinin (Z or BA) with different auxins (IAA or NAA). Furthermore, Palla et al. [68] studied *in vitro* culture and rooting of *D. virginiana* L. from nodal root explants by using several phytohormones and culture media. The authors indicated that the presence of auxins was not essential but slightly accelerated the organogenic callus formation and organogenesis. Cryopreservation is recognized as having the distinctive advantage of allowing long-term conservation with minimum space and maintenance [69]. Matsumoto et al. [70] studied cryopreservation of persimmon (*D. kaki* Thunb.) by vitrification of dormant shoot tips. The authors recorded that using dormant shoot tips was promising as a routine method for the cryopreservation of *Diospyros* germplasm.

After the great progress in *in vitro* regeneration of plants from protoplasts, several researches focused on plant somatic hybridization which allows combining protoplasts from different cultivars, species or genera for variety improvement [71]. Tao et al. [72] reported plant regeneration from callus protoplast of *D. kaki*. They used callus as the protoplast source derived from leaf primordia excised from dormant winter buds of adult Japanese persimmon (*D. kaki* L. cv. Jiro) for plant regeneration. Tamura et al. [73] studied protoplast culture and plant regeneration of *D. kaki* L. and reported that plantlets could be obtained from the protoplast-derived calli. Tamura et al. [74] indicated that somatic hybrids of Japanese persimmon (*D. kaki* L.) were obtained by electrofusion of protoplasts. Callus protoplasts of Jiro and Suruga were fused electrically and cultured in modified KM8p medium using agarose-bead culture. The authors recorded that the fused products had the dodecaploid chromosome number of around  $2n = 180$ , which is twice the number of parental plants ( $2n = 90 \times = 15$ ). In addition, Tamura et al. [75] recorded interspecific somatic hybrids between *D. glandulosa* ( $2n = 2 \times = 30$ ) and *D. kaki* cv. Jiro ( $2n = 6 \times = 90$ ) produced by electrofusion of protoplasts. Colchicine treatment of actively dividing cells can induce chromosome doubling and has been used to make plants with



doubled chromosome number. Colchicine treatment to a protoplast at the very beginning of its division could be one method to overcome the problem because plants can be regenerated from a single cell with doubled chromosome number. Tamura et al. [74] reported production of dodecaploid plants of Japanese persimmon by colchicine treatment of protoplasts.

Improved genomic research and resources, in recent years, have resulted in the development of screening tools via marker-assisted selection (MAS). Using MAS has led to more efficient selections and has increased the efficiency in persimmon breeding programs hastening the release of new cultivar. In order to obtain PCNA offspring in breeding programs, the parental materials considered for choosing the cross combinations have to be PCNA type regarding the inheritance of astringency. However, repeated crosses among PCNA cultivars/selections has led to inbreeding depression for tree vigor, productivity and fruit weight [36]. In these situations, marker-assisted selection should be developed for selecting PCNA offspring efficiently. Recently, Kanzaki et al. [47] have developed molecular markers associated with the trait of natural astringency loss in persimmon fruit and the markers are practically useful in persimmon breeding programs. In addition, Mitani et al. [76] studied if the SCAR markers could reliably distinguish PCNA and non-PCNA genotypes in a large number of offspring derived from backcross between 'Taigetsu' and PCNA 'Kanshu'. The authors indicated that PCNA offspring can be selected by two PCR primers in the progeny derived from 'Taigetsu' × 'Kanshu'. Yonemori et al. [77] reported molecular marker for selecting PCNA type persimmon progenies at the juvenile stage. Yonemori et al. [77] constructed a reliable PCR marker for selecting PCNA type offspring among breeding population of persimmon. In addition Kanzaki et al. [47] and Mitani et al. [76] reported that SCAR markers can practically be used in application of marker-assisted selection in persimmon breeding.

Genetic transformation is also an alternative technique for persimmon genetic improvement. Transgenic persimmon cultivars thus produced have potential for commercial success and grower acceptance because the unique genetic constitution of the cultivars has not been disturbed. Tao et al. [78] reported genetic transformation of persimmon by *Agrobacterium rhizogenes*. Phenotypic alterations such as dwarfness and decrease in rooting ability were observed in the transformants. In addition, Gao et al. [79] transformed 'Jiro' persimmon with Arabidopsis FT gene (*AtFT*) and *PmTFL1* gene, a *Prunus mume* ortholog of Arabidopsis *TFL1* gene. The authors indicated that the *PmTFL1* transgenic *in vitro* shoots did not show a different appearance compared with non-transformed 'Jiro' shoots, however, the *AtFT* transgenic shoots indicated a 'bushy' phenotype having the short internodes.

## 9. Conclusions

Persimmon can adapt to a wide range of climatic conditions. Production in many countries having subtropical and tropical climates satisfies domestic demand and creates new export opportunities. Increasing the world persimmon production has been very successful since 1995. Recently, the applications of the technique for removing astringency without losing fruit firmness have been significantly promoted to increase the production. It is expected that the production will significantly increase over the next few decades.

Selection breeding is the most common breeding technique in persimmon because persimmon breeding is mainly hindered by its high ploidy and by its complex sex expression. Fuyu, Hachiya, Hiratatenashi, Izu, Jiro and Saijo cultivars which are extensively growing all over the world were selected from shoots of bud sports in Japan. We should continue the screening of plants coming from spontaneous mutations. Hybridization method can also be used in persimmon breeding. Hybridization studies among *D. kaki* in Japan have led to the release of a lot of PCNA cultivars which ripen at different times. There is also a unique hybrid of Asian and American persimmon. In Spain, studies on induced mutation have also led to the development of new cultivars.

There are a number of collections including many accessions in institutions of the various persimmon growing countries. The morphological and molecular characterization of all the persimmon accessions needs to be achieved. The information developed from this will be highly beneficial for screening against biotic and abiotic stress factors. Genomics and transcriptomic resources need to be developed for persimmon. It will also lead to the development of new and improved cultivars of persimmon.

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