Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1241036

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 6 - Issue 3: 262-268 / May 2023

DETERMINATION OF POLLINATOR CHARACTERISTICS OF SOME HAZELNUT GENOTYPES

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Abstract: This research was carried out in Karasu, Kocaali and Arifiye districts of Sakarya Province to determine the suitable pollinators for Çakıldak hazelnut cultivar (*Corylus avellana*) in 2021-2022. In the study, 27 genotypes that were late male flowering, formed a large amount of catkins, had high pollen quality, had round nut shape and short husk length were examined. Pollen viability was detected according to 2, 3, 5,-Triphenyl Tetrazolium Chloride (TTC). Pollen viability of the genotypes was ranged from 22.3% to 93.7% according to the TTC method. According to the agar method, pollen germination rate was determined between 15.6% and 78.1% at 20% sucrose concentration containing 1% agar. 89% of the genotypes were round, 11% were in the oblong nut group. It was determined that most of them amount of male inflorescences (catkins) depending on the tree crown volume and age. T-22KRS02, T-22KRS03, T-22KRS07, T-22KRS08, T-22KRS09, T-22KRS10, T-22KRS11, T-22KCL11, T-22KCL14, T-22KCL16 and Mincane were selected to be evaluated in the second phase of the study.

Keywords: Climate change, *Corylus avellana*, Dichogamy, Pollinator, Yield, Quality

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Received: January 23, 2023
Accepted: April 12, 2023
Published: May 01, 2023
Cite as: Balik Hİ, Demir T, Beyhan Ö. 2023. Determination of pollinator characteristics of some hazelnut genotypes. BSJ Agri, 6(3): 262-268.

1. Introduction

Hazelnut *(Corylus avellana* L.) is a fruit species cultivated in temperate climate zones, whose production area, amount and trade continue to increase day by day. Türkiye constitutes 72.3% of the world hazelnut production area with a production area of 734,538 hectares. With a production of 665,000 tons, it is the most important hazelnut producer country, realizing 62% of the world hazelnut production. Türkiye is followed by Italy, USA, Azerbaijan and Chile. Hazelnut (*Corylus avellana* L.) is monoecious, self and cross-compatible and wind-pollinated. Incompatibility in hazelnut is sporophytic-type controlled by a single locus and S alleles (Thompson, 1979). As a result of the studies carried out to the present day, 33 S alleles have been determined (Mehlenbacher, 2014). It was determined that allele genes in the pistil were co-dominant and those in the pollen were dominant or co-dominant (Mehlenbacher ve Thompson, 1988).

Investigation of the periodic biological events affected by the environment, especially the temperature changes caused by weather and climate in plants, is called phenology (Crepinsek et al., 2012). Bud break, harvest, male and female flowering time are phenologically important stages in hazelnut cultivation. Unlike many temperate fruit species, hazelnuts bloom in winter. Male and female flowers may vary depending on the variety, altitude and year (Beyhan, 2000). Although hazelnut cultivars are partially self-incompatible, studies have shown that cross pollination increases nut set and nut quality (Balık and Beyhan 2019a; Balık and Beyhan 2019b; Balık and Beyhan 2020; Balık and Beyhan 2021; Fatahi et al., 2014; Hosseinpour et al., 2015; Javadi and Gheshlaghi, 2006). Cross pollination is required for high yield in hazelnut and at least two pollinators are recommended. Pollinators should not show incompatibility with the main cultivar, pollen quality should be high, pollen distribution time should be as long as possible (Hampson et al., 1992).

There are two basic approaches in terms of nut set in hazelnut. The first is pollen-stigma compatibility, and the other is phenological male and female flowering at the same time. In recent years, as a result of the adverse effects of climate changes, high temperatures in the Black Sea Region in autumn and winter cause male flowers to bloom much earlier in hazelnut cultivars, and female and male flowering do not coincide enough to provide sufficient nut set (Balık and Beyhan, 2019a; Beyhan and Marangoz, 2007). Cross-pollination is necessary due to dichogamy and incompatibility in hazelnut. Male flowering is usually in January in Turkish hazelnut cultivars. Due to global warming, it is noteworthy that in recent years, male flowering has taken place earlier and may be as early as 1 month. However, pollination does not occur during this period as female flowers have not receptive yet. Therefore, there is a need for pollinators that bloom during the period when female flowers are receptive. In hazelnut, it is desired that the pollinators bloom as late as possible and the pollen distribution takes place in a long period. In addition, pollinators should have high viability and germination rates and should not show incompatibility (Beyhan, 2000; Balık and Beyhan, 2019b).

In this study, the potential of genotypes with high pollen quality, which do not show incompatibility, flowering at the same time with the main cultivars, as pollinator Karasu, Kocaali and Arifiye districts of Sakarya was investigated.

2. Material and Methods

The plant material of the study consists of 27 genotypes selected in Karasu, Kocaali and Arifiye districts of Sakarya province (Table 1).

Table 1. Location Information of Genotypes

No	Genotypes	District	Village	Altitude (m)
1	T-22KRS01	Karasu	Kuyumculu	50
2	T-22KRS02	Karasu	Kuyumculu	81
3	T-22KRS03	Karasu	Kuyumculu	65
4	T-22KRS04	Karasu	Salıkkaya	200
5	T-22KRS05	Karasu	Salıkkaya	232
6	T-22KRS06	Karasu	Salıkkaya-Kirazlı	249
7	T-22KRS07	Karasu	Kirazlı	160
8	T-22KRS08	Karasu	Kirazlı	220
9	T-22KRS09	Karasu	Kirazlı	206
10	T-22KRS10	Karasu	Kirazlı	195
11	T-22KRS11	Karasu	Kirazlı	190
12	T-22KCL03	Kocaali	Gümüşoluk-Demiraçma	103
13	T-22KCL04	Kocaali	Gümüşoluk	202
14	T-22KCL05	Kocaali	Gümüşoluk	211
15	T-22KCL06	Kocaali	Açmabaşı	120
16	T-22KCL07	Kocaali	Açmabaşı	175
17	T-22KCL08	Kocaali	Açmabaşı	209
18	T-22KCL11	Kocaali	Kızılüzüm	658
19	T-22KCL12	Kocaali	Kestanepınarı	382
20	T-22KCL13	Kocaali	Kestanepınarı	385
21	T-22KCL14	Kocaali	Chamber of Agriculture	160
22	T-22KCL15	Kocaali	Açmabaşı	156
23	T-22KCL16	Kocaali	Açmabaşı	150
24	Allahverdi	Arifiye	SUBU Faculty of Agriculture Research Center	30
25	Kalınkara	Kocaali	Chamber of Agriculture	160
26	Mincane	Kocaali	Chamber of Agriculture	160
27	Okay 28	Arifiye	SUBU Faculty of Agriculture Research Center	30

In the selection of genotypes, it was taken as a criterion that the male flowering was later than the standard cultivars. Since January 2022, phenology has been followed in both districts on the basis of cultivar and altitude, and genotypes that still continue to bloom after the end of male flowering in standard cultivars have been determined. Catkins were taken from the genotypes and viability and germination tests were performed on the pollen obtained after they were kept at room temperature for 24 hours. While 2, 3, 5,-Triphenyl Tetrazolium Chloride (TTC) method was applied in pollen viability test (Figure 1), pollen germination was determined at 20% sucrose concentration containing 1% agar compared to agar method (Figure 2).

Figure 1. Pollen viability test in hazelnut (viable pollen and dead pollen).

Figure 2. View of the pollen germination in hazelnut (20% sucrose solution).

In addition, the husk and nut characteristics of the genotypes were also evaluated. Evaluation of genotypes was made according to Weighed Rating Method (Table 2).

TTC method was used to determine pollen viability. 2 h after the application of TTC test, which was carried out in daylight, counting under the light microscope (Zeiss Axiolab 5, 40×) was made, and the red-stained pollen were considered as alive and the unstained ones as dead.

Agar method was used to determine of the pollen germination (Beyhan and Odabaş, 1997). It was kept in the test cabinet (Microtest Mit 500) at 20 °C for 36 h under 65% relative humidity conditions. Pollens with a length of pollen tube greater than the diameter of pollen were considered germinated under the light microscope (Zeiss Axiolab 5, 40×).

Phenological characters were determined according to Çalışkan and Çetiner (1997). Genotypes are grouped as less, medium, and high in male inflorescences, taking into account the size of the crown, age, and growth pattern.

Table 2. Properties evaluated in the Weighed Rating Method

Traits	Relative Score	Categories	Categories Range	Point
Pollen viability (%)	25	Very high	79<	9
		High	65-78	7
		Medium	51-64	5
		Low	37-50	3
		Very low	<36	1
Pollen germination (%)	15	Very high	66<	9
		High	53-65	7
		Medium	41-52	5
		Low	28-40	3
		Very low	<27	1
Amount of catkins	25	More		5
		Maedium		3
		Low		1
Nut shape	25	Globular		5
		Pointed		3
		Cylindirical		1
Husk length	10	Taller than nut		1
		Equal to nut		3
		Shorter than nut		5

3. Results and Discussion

The pollen viability of the genotypes was determined between 22.3% and 93.7% (Table 3). Most of the genotypes are included in the wild hazelnut population and it was planted in orchards due to its pollinating properties. Due to the low yield and nut quality of these genotypes, it is seen that cultural management processes such as pruning and fertilization are not applied. It is considered that the lack of cultural practices and the wide genetic variation cause the difference in pollen viability rates of genotypes to be high. As a matter of fact, Beyhan (2000) stated that pollen quality in hazelnut can vary depending on the variety, year, cultural practices and ecology. Beyhan and Odabaş (1995) determined pollen viability rate as 89% in Tombul, 78% in Palaz, 72% in Çakıldak, 76% in Sivri and 88% in Kalınkara. Balık and Beyhan (2019c) determined that pollen viability rate in ranged from 1.61% (Yassı Badem) to 71.03% (Allahverdi).

Pollen germination rate of genotypes was determined between 15.6-78.1% (Table 3). Beyhan and Odabaş (1995) determined pollen germination rates of Turkish hazelnut cultivars between 27-76% in pollen germination tests performed at different sucrose concentrations. In the same study, pollen germination rates were determined as 69% in Tombul, 49% in Palaz, 60% in Çakıldak, 63% in Sivri and 52% in Kalınkara at 25% sucrose concentration, where pollen germination rates were the highest. The pollen germination rates determined in the research were similar to the literature. However, it was noteworthy that pollen germination rates of genotypes were lower than pollen viability rates. Fatahi et al. (2014) emphasized that pollen quality was changed to according to cultivars, and pollen germination rates in the examined cultivars were determined above 60%. Stösser et al. (1996) reported that pollen germination tests performed in vitro, as well as climatic conditions, may be affected by pollen collection time and pollen storage conditions. Moore and Janick (1983) emphasized that pollen density, germination density and pH were effective on pollen germination rate under in vitro conditions. Mert (2009) determined that pollen germination rate increased as the temperature increased in the walnut cultivars. Beyhan and Odabaş (1995) determined that not all pollen that was determined to be viable germinated and this situation was more pronounced in some cultivars. It was emphasized that it would be more accurate to determine the pollen quality of cultivars by viability test, because variable external factors such as ambient humidity, temperature and the properties of the substances used as substrate were effective in pollen germination. When pollen viability test results were compared with germination results, it was seen that higher rates were obtained in viability tests. On the other hand, it was stated that there was a strong relationship between pollen viability and germination rate (Novara et al., 2017).

Table 3. Pollen viability and germination of genotypes, amount of catkins, nut shape, husk length values and total weighted rating scores.

G	PV (%)	Point	PG (%)	Point	AC	Point	Nut shape	Point	Husk length	Point	TWRS	Evaluation
T-22KRS01	93.5	9	51.6	5	Much	5	Cylindrical	1	Short	5	500	
T-22KRS02	86.7	9	63.9	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS03	89.7	9	41.3	5	Much	5	Globular	5	Short	5	600	Chosen
T-22KRS04	92	9	36	3	Much	5	Globular	5	Equal	3	550	
T-22KRS05	60.8	5	42.6	5	Much	5	Globular	5	Equal	3	480	
T-22KRS06	76.8	7	29.5	3	Much	5	Globular	5	Tall	1	480	
T-22KRS07	71.3	7	72.3	9	Much	5	Globular	5	Tall	1	570	Chosen
T-22KRS08	89.7	9	58.9	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS09	85	9	57	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS10	93.7	9	65.4	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS11	93.7	9	59.7	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KCL03	90.3	9	36.9	3	Much	5	Cylindrical	1	Short	5	470	
T-22KCL04	65	7	52.4	5	Much	5	Cylindrical	1	Tall	1	410	
T-22KCL05	78.3	7	57.6	7	Much	5	Globular	5	Equal	3	560	
T-22KCL06	90.7	9	30.5	3	Much	5	Globular	5	Tall	1	530	
T-22KCL07	73.3	7	58.4	7	Much	5	Globular	5	Tall	1	540	
T-22KCL08	70	7	54.5	7	Much	5	Globular	5	Tall	1	540	
T-22KCL11	84	9	53.4	7	Much	5	Globular	5	Short	5	630	Chosen
T-22KCL12	91.7	9	34.2	3	Much	5	Globular	5	Equal	3	550	
T-22KCL13	77.3	7	32.4	3	Much	5	Globular	5	Short	5	520	
T-22KCL14	87	9	78.1	9	Much	5	Globular	5	Tall	1	620	Chosen
T-22KCL15	43	3	28.3	3	Much	5	Globular	5	Tall	1	380	
T-22KCL16	92.3	9	71.7	9	Much	5	Globular	5	Equal	3	640	Chosen
Allahverdi	81.3	9	70.8	9	Much	3	Globular	5	Tall	1	570	
Kalınkara	22.3	1	15.6	1	Much	3	Globular	5	Tall	1	300	
Mincane	88.7	9	75.8	9	Medium	3	Globular	5	Tall	1	570	Chosen
Okay 28	43.7	3	67.3	9	Few	1	Globular	5	Tall	1	370	

G= genotypes, PV= pollen viability, AC= amount of catkins, PG= pollen germination, TWRS= total weighted rating scores

Male inflorescences in hazelnuts begin to appear on the ends and sides of seasonal shoots in July. There are 150-200 male flowers in catkins. On the other hand, more than one catkin may form from one bud. There are 4 male organs in each bract leaf of catkins. There are 2 anthers in each of the male organs. Cross pollination is required for high nut set in hazelnut. At least two pollinator cultivars are recommended. Pollinators should not show incompatibility, produce a large number of catkins, pollen viability should be high, pollen shading should be as long as possible (Hampson et al., 1992). In our study, it was observed that although the genotypes were grown in different ecological conditions, they formed quite a lot of catkins depending on the plant age and tree volume (Figure 3). However, the fact that these genotypes have a high amount of catkins does not mean that they are ideal pollinators.

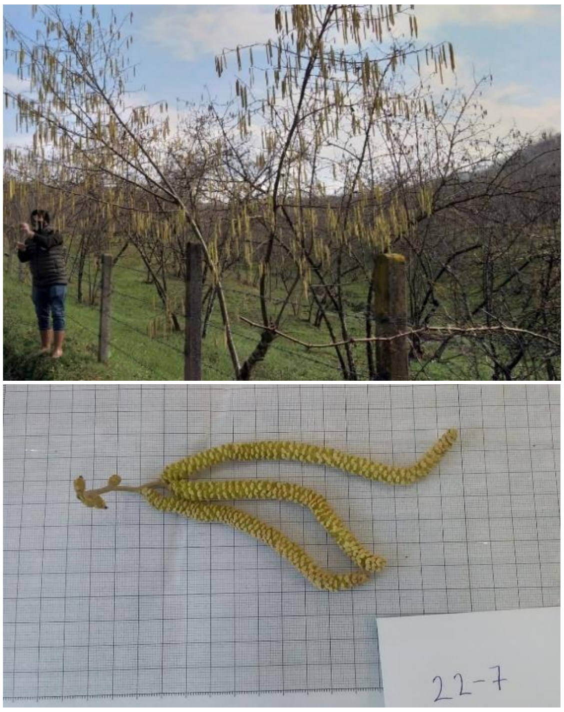

Figure 3. Plant and catkins image of pollinator genotypes

It has been determined that the genotypes are in two groups as the nut shapes are cylindrical and globular (Figure 4). Turkish hazelnut cultivars are divided into three groups according to nut shape: globular (Ex:

Tombul, Palaz, Çakıldak, Kalınkara), conical (Ex: Acı, Sivri) and cylindrical (Ex: Yuvarlak Badem, Yassı Badem) (Balık et al., 2016). Balık (2018), determined that pollinators cause a change in nut shape in hazelnut. Çetiner et al. (1984) stated that Sivri and İncekara cultivars, which provide the highest level of nut set, but it may cause deterioration of homogeneity and product quality in orchards due to the sharp nut shape. In addition, it has been stated that the nut shape may deteriorate in case of an increase in the number of nuts in cluster, which is a type of hazelnut and can change depending on climatic conditions and maintenance conditions (Balık et al. 2014). Owais (2014) reported that pollinators cause a significant change in kernel shape in almonds. On the other hand, it has been noted that self-pollination in almonds causes irregular fruit shape (Graselly and Olivier, 1988; Torre Grossa et al., 1994) and abortive kernel (Torre Grossa et al., 1994).

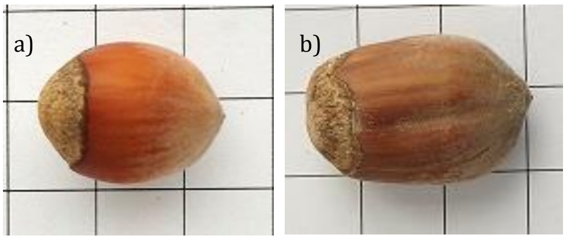

Figure 4. Globular (a) and cylindrical (b) nut shape in Turkish hazelnut cultivar (Balık et al., 2016).

Husk image of pollinator genotypes is presented in Figure 5. The husk length in hazelnut was compared with the nut and grouped into three categories as shorter than the nut, equal to the nut and longer than the nut (UPOV, 2022). While the husk length was longer than the nut in 17 of the genotypes examined, it was determined that the husk and nut were equal in length in 5 genotypes, and the husk length was shorter than the nut in 5 genotypes. It is seen that the cultivars grown especially in countries where mechanical harvesting is applied have a short and loose husk structure that surrounds the nut. Turkish hazelnut cultivars, especially due to the topographic structure of the Eastern Black Sea Region, where they were cultivated for the first time, have resulted in the surviving of the varieties in which the husk is long and tightly wraps the nut, as a result of a conscious selection (Balık et al., 2021).

Figure 5. Husk image of pollinator genotypes

4. Conclusions

This research was carried out to determine the ideal pollinators for Çakıldak cultivar, which was found to have pollination problems due to climate change. For this purpose, 27 genotypes that were still in the male flowering stage despite the completion of male flowering in standard hazelnut cultivars were examined. Pollen viability of genotypes was determined as 22.3-93.7%, pollen germination between 15.6-78.1%. It is considered that the variation in pollen viability and germination rates is due to the different altitude, vertical and ecological conditions in the locations where the genotypes are determined, and the genetic variation is high due to the fact that wild hazelnut types are included in the seedling population.

The changes in the nut and kernel characteristics of the pollinators in hazelnut are called xenia and metaxenia (Balık, 2018). Since pollinators cause changes in nut shape, the genotypes examined in our research were also evaluated according to nutshape and was scored with 25 relative points in the weighted grading method. 89% of the genotypes examined were globular and 11% were in the cylindrical nut group. Globular shaped hazelnuts are preferred in the world hazelnut markets.

In hazelnut cultivation, it is expected that pollinators do not incompatibility and dichogamy, have a globular nut shape, high pollen quality, as well as producing a large amount of catkins every year. It was determined that most of the genotypes examined in our study formed a 'many' amount of catkins depending on the tree crown volume and age.

The origin of hazelnut is Anatolia and the majority of production is done in the Black Sea Region. Hazelnut cultivars, which have been produced for thousands of years, have reached the present stage as a result of selection. The rough and sloping land of the Black Sea Region has caused the producers to care about the cultivars with a long husk, which tightly surrounds the fruit, and the selection of these cultivars has caused the production to become widespread. For this reason, Turkish hazelnut cultivars have long husk characteristics that tightly wrap the nut. However, these characteristics of the cultivars make mechanical harvesting difficulty. Harvest labor, which has a 40% share in the hazelnut production cost, is getting harder every year due to the migration of the young population to the cities, the aging of the population in the villages, and the high cost of recruiting harvest workers from other regions and abroad. For this reason, it is necessary to establish the orchards with a training system and cultivars suitable for mechanical harvest. It is important to use pollinators with short husk in terms of suitability for mechanical harvesting.

Çakıldak is the most widely grown hazelnut cultivar in Karasu and Kocaali district. The cultivation rate of Çakıldak in these districts is 60% on average, and the high kernel ratio and yield and the fact that it is not damaged by late spring frosts due to late bud burst causes Çakıldak to become widespread rapidly. For this reason, efficient and high quality production of Çakıldak is very important in terms of producer comfort. It has been determined that the pollinators are not included in the orchards established with the Çakıldak, the male and female flowering times differ due to global warming, and the lack of pollination due to the increase in the dichogamy degree causes yield losses.

As a result of this research carried out in order to reduce the yield loss due to the lack of pollination in Çakıldak; among the genotypes with high pollen quality, high amount of catkins, short husk length and globular nut shape, the total weighted rating score is 570 and above T-22KRS02, T-22KRS03, T-22KRS07, T-22KRS08, T-22KRS09, T-22KRS10, T-22KRS11, T-22KCL11, T-22KCL14, T-22KCL16 and Mincane were selected for evaluation in the second phase of the investigation.

Whether the genotypes are suitable pollinators for Çakıldak should be examined by following controlled hybridizations (nut set) and development of the pollen tube in style (incompatibility) under fluorescence microscope. Promising pollinator cultivar candidates identified as a result of these studies should be registered, a large number of plants should be produced with tissue culture, and the use of producers should be expanded.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	H.İ.B	T.D	Ö.B
C	50	25	25
D	50	25	25
S	50	25	25
DCP	100		
DAI	50	25	25
L	50	25	25
W	50	25	25
CR	50	25	25
SR	50	25	25
PM	80	10	10
FA	50	25	25

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgement

This research was supported by Sakarya University of Applied Sciences Scientific Research Projects Coordinatorship within the scope of project number 041-2021.

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