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**Research Article**

# Pollen compatibility in Turkish hazelnut cultivars

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**ABSTRACT**

This study was conducted with the aim of determining the pollen incompatibility levels of some important hazelnut cultivars. Tombul, Palaz, Çakıldak, Foşa and Allahverdi were used as main cultivars and Tombul, Palaz, Çakıldak, Foşa, Allahverdi, Sivri, Kalınkara and Yassı Badem were used as a pollinizer cultivars. When the effects of pollinizers on pollen compatibility and flowering times were evaluated together; it was defined that Allahverdi for Tombul, Foşa for Palaz, Tombul for Çakıldak, Çakıldak and Allahverdi for Foşa, and Foşa are the best pollinizer for Allahverdi.

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## 1. Introduction

Pollination and fertilization are essential for nut set in hazelnuts. Male and female flowers blooming at different times. Blossoming varies depending on the ecology, cultivar and years of the same cultivar (Beyhan, 2000). Start of blooming and blooming times are related to climatic factors and especially temperature. Blooming starts early in regions with warm climate winters. High temperatures in autumn and winter in recent years cause male flowers to bloom earlier than female flowers and dichogamy degrees of some cultivars are increasing.

There is a self-incompatibility and cross-incompatibility in hazelnut. This incompatibility is determined sporophytically by a single locus with multiple alleles (Ives et al., 2014). As a result of the studies carried out to the present day, 33 S alleles have been determined (Mehlenbacher, 2014). Hazelnuts are diploid (2n=2x=22), and most cultivars are heterozygous at the S-locus. Both alleles are expressed in the stigmas, but often only one is expressed in the pollen because of dominance (Ives et al., 2014). Electron microscopy has shown the presence of incompatibility regions on the stigma in hazelnuts. In case of incompatibility, pollen germination of the stigma is delayed and the pollen pipe cannot enter the style.

Dichogamy and incompatibility mechanisms in hazelnuts require the use of pollinizer cultivars. It has been shown in many studies that cross pollination significantly increases nut set. Cross pollination is required for sufficient nut set in hazelnut. At least two pollinizer cultivars are recommended. Pollinizer cultivars should not be in incompatibility with the main cultivars, pollen viability should be high (Hampson et.al, 1992).

In sporophytic self-incompatibility, all of the pollens of the plant behave similarly regardless of the S allele and the dispute is controlled by sporofit. For example, pollen having an S1 or S2 allele from a plant with S1S2 alleles, S1 acts as S1 if dominant, and S2 as S2 if dominant. In other words, even the presence of one allele of the style tissue in the sporophytic tissue of the male parent will cause all pollen of this plant to be ineffective against the style. Therefore, plants carrying the S1S2 allele S1S4, S1S5, S2S3, S2S4, S2S5 and so on. 100% incompatible with plants carrying the allele, plants with S3S4 and S3S5 alleles will not be incompatible (Ünal, 2009).

It is very important that the hazelnut producers have sufficient information about the nut set problems that will arise in the orchard establishment due to self or cross incompatibility. For this reason, it is necessary to determine the incompatibility of hazelnut cultivars and to recommend the pollinizers for self-incompatible cultivars to the hazelnut producers. Determination of the nut set by self-pollination and crossings, observation of pollen tube growth in the tube by using fluorescence microscopy technique, and incompatibility can be determined by molecular methods (Erdem et. al, 2013; Karakaş and Beyhan, 2012).

This study was conducted to determine the self and cross-compatibility levels of Turkish hazelnut cultivars.

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## 2. Materials and methods

This study was conducted under ecological conditions of Giresun province in the years 2015 and 2016. Experimental plots were constructed in multi-bushed system (ocak system) at 3x3 m spacing in 1994. Tombul, Palaz, Çakıldak, Foşa and Allahverdi were used as main cultivar. Self-pollination and open-pollination were practiced. These cultivars were controlled-pollinated with Sivri, Kalınkara and Yassı Badem cultivars. Experiments were conducted in randomized blocks design with 3 replications and with 3 plants (bushes) in each replicate.

In plants of main cultivars to be controlled-hybridized, male flowers (catkins) were removed as specified by Erdoğan and Mehlenbacher (1997).

Plants of main cultivars were surrounded by 4 m high and 4 m wide iron constructions and entire plant was encapsulated within this framework and covered with tyvek.

When the catkins of pollinizer cultivars started to be elongated, they were carefully cut together with the shoot bearing catkins, they were placed in water-filled glass jars and kept at room temperature for 24 hours. Each cultivar was kept in different room to prevent interactions of pollens. Following 24 hours, catkins were shaken over a black paper, pollens were sieved through 125 µ sieve and transferred to preservation cups. Pollens were preserved in a deep freezer at -18 °C until the time of crossings.

When the female flowers of main cultivars turned into receptive status, pollens preserved in deep freezer were used to perfom hybridizations. Stigmatic styles can accept pollens as soon as they went out of florets, but it was waited until these styles got a shiny red color for an effective pollination. The pollens stored in preservation cups in a deep freezer were transferred to eppendorph tubes at the day of pollination. Lid of eppendorph tube was opened, the tube turnd upside down and flower pollens were taken over the index finder. Then artificial pollination was performed through touching to styles of the florets with the index finger. One week after hydridizations, browning was observed in floret styles.

To determine the effects of pollinator types on nut set, 3 plants (bushes) orienting different directions were selected from 3 different multibush system (ocak) for each hybridization combination and number of female flowers over which hybridizations were performed was recorded.

To evaluate the level of self-and cross incompatibility, the index of pollen incompatibility was applied Hosseinpour et al. (2015). Index of pollen incompatibility is the ratio of nut set after self-cross pollination to nut set after open-pollination, as a potential compatible cross. When the ratio is $\leq 0.2$ the cross is incompatible, 0.2-1 is partially compatible and $\geq 1$ is completely compatible.

Phenological characteristics such as male and female flowering times and vegetative bud break time were determined by using Calıskan and Cetiner (1997).

Experimental data were subjected to statistical analyses with the aid of SAS Version 9.1 software. Significant means were compared with the aid of Duncan's multiple range test at 5 % level ($P<0.05$). Data were subjected to analysis of variance separately for each year. In 2015, no data was obtained in Palaz x Kalınkara hybridization combination.

## 3. Results and discussion

In both years of the experiment, Tombul was protogyn, while Palaz, Çakıldak and Sivri cultivars were protandry, Foşa protogyn-homogamy, Allahverdi and Yassı Badem were homogamy. Kalınkara is protandry in 2015 and protogyn in 2016. Blossoming varies depending on the ecology, cultivar and years of the same cultivar (Beyhan, 2000).

While the earliest cultivars of male flowering in 2015 were Sivri, Kalınkara and Yassı Badem, in 2016 it was determined that Sivri and Yassı Badem were the earliest flowering cultivars. Male flowering was the latest in two years of the experiment in Tombul.

Male flowering period of cultivars in 2015; 8 days in Tombul, 38 days in Palaz, 16 days in Çakıldak, 21 days in Foşa, 41 days in Allahverdi, 32 days in Sivri, 43 days in Kalınkara and 22 days in Yassı Badem. In 2016, male flowering periods of the cultivars were determined as 11 days, 35 days, 19 days, 24 days, 30 days, 34 days, 25 days and 34 days, respectively. In the 2015-2016 flowering period, male flowering period was found to be longer in Tombul, Çakıldak, Foşa, Sivri and Yassı Badem cultivars than in the previous year (Figure 1).

In both years of the experiment, styles were first seen in Kalınkara and Yassı Badem cultivars. It was determined that female flowering in Çakıldak in 2015 and Palaz and Çakıldak in 2016 at the latest. Receptivity of female flowers in 2015 is 25 days in Tombul, 23 days in Palaz, 26 days in Çakıldak, 42 days in Foşa, 55 days in Allahverdi, 33 days in Sivri, 38 days in Kalınkara and Yassı Badem recorded as 34 days. In 2016, it was determined as 24 days, 21 days, 24 days, 28 days, 35 days, 30 days, 50 days and 30 days respectively. In the 2015-2016 flowering season, the receptivity of female flowers was longer in Kalınkara cultivar than in the previous year and in other cultivars was shortened (Figure 1).

In 2015, effects of pollination treatments on nut set were found to be significant in all varieties except for Allahverdi. In 2016, effects of treatments on fruit set of all varieties were found to be significant ($P<0.05$). In the first year of the experiments (2015), the lowest nut set values were observed in self-pollination treatments of Palaz and Çakıldak cultivars. In 2016, besides Palaz and Çakıldak cultivars, the lowest nut set values were also observed in self-pollination treatments of Tombul and Allahverdi cultivars. Therefore, it can be stated that cross pollinations increased nut sets in both years (Table 1-2). Fatahi et al. (2014) indicated that cross pollinations significantly increased nut set of hazelnut cultivars. De Nettancourt (1977), indicated that low nut set of self-pollination treatments was the indicator of self-incompatibility. Çakır and Genç (1971) reported nut set as 27.5% for self-pollination of Tombul cultivar, 52% for Tombul x Palaz pollination treatment, 51% for Palaz x Yabani Sivri pollination treatment, 46% for Çakıldak x Tombul pollination combination and indicated that cross pollinations increased nut set of hazelnuts.

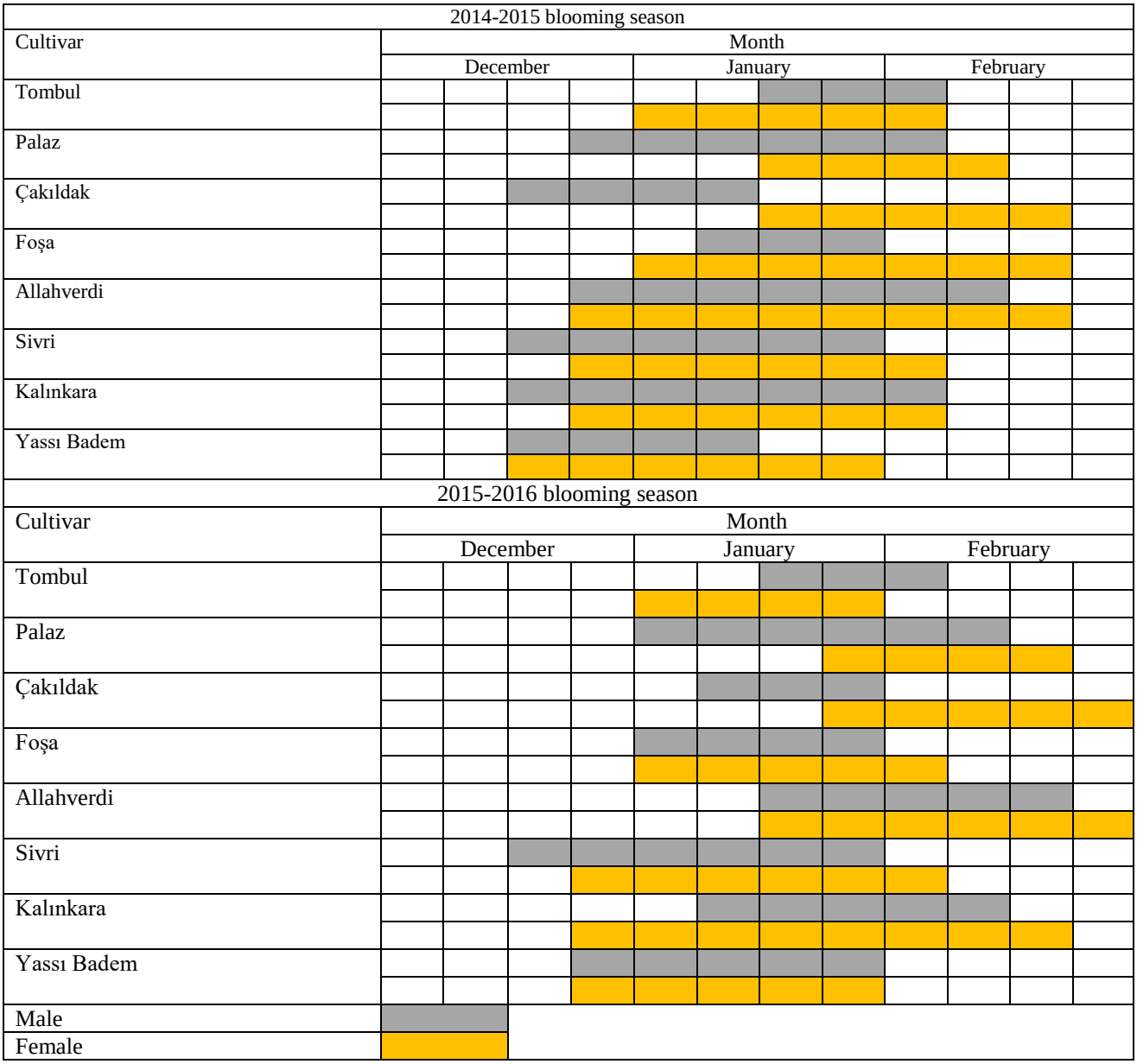


**Figure 1**. Male and female blooming of hazelnut cultivars in 2014-2015 and 2015-2016 flowering seasons

On the other hand, Xie and Liu (2014), pointed out that fertile efficiency increased when the pollen vigor was high. Erdoğan and Mehlenbacher (2001), indicated that low nut set of self-pollination treatments in hazelnut mostly resulted from self-incompatibility and defined the indicators of incompatibility as low germination ratios of pollen in stigma, short and curly nature of pollen tube. Despite the incompatibility in several combinations, low nut sets in field experiments were attributed to pollen germination and development of pollen tube, as well as failures in pollen germination and intrusion of pollen tube into style, damage of pollen tube in style with the impact of pollination, embryo abortion, endosperm defects and hybrid deterioration as indicated by Lield and Anderson (1993). Olsen et al. (2000), indicated that there was self and cross incompatibility in hazelnut and such incompatibilities were sporophytic type controlled by a single locus and S alleles. Beyhan and Marangoz (2007), indicated the reasons of cluster drops outs as genetic structure, periodicity, pollen source, incompatibility, cultural practices and environmental conditions.

In 2015, when it was pollinated with Foşa and Yassı Badem in the Tombul cultivar, it was evaluated that there was no incompatibility, in other applications there has been a partial compatibility. In contrast, there were no incompatibility in Çakıldak and Allahverdi pollination applications, other applications have been considered partially compatible in 2016 (Table 3-4).

It was determined that there was no incompatibility in Tombul, Palaz, Foşa, Sivri, Kalınkara and Yassı Badem pollination applications in both years of the experiment in the Çakıldak cultivar. While there was no incompatibility in Allahverdi pollination application in 2015, the same pollination application in 2016 was considered partially compatible. On the other hand, there is a partial compatible in both years of the experiment in self-pollination (Table 3-

4).

**Table 1**. Effect of pollen source on nut set of hazelnut cultivars in 2015 (%)

| Pollinizer Cultivars | Main Cultivars | | | | |
|---|---|---|---|---|---|
| | Tombul | Palaz | Çakıldak | Foşa | Allahverdi |
| Tombul | 62.33 ab* | 57.47 a | 69.15 ab | 24.39 b | 79.02 |
| Palaz | 46.48 cd | 7.49 b | 74.52 a | 8.29 b | 63.82 |
| Çakıldak | 55.81 bc | 46.37 a | 27.10 c | 65.79 a | 64.97 |
| Foşa | 70.14 a | 61.64 a | 63.61 ab | 66.17 a | 67.52 |
| Allahverdi | 49.94 cd | 58.40 a | 68.54 ab | 11.27 b | 64.26 |
| Sivri | 45.68 d | 21.96 b | 63.86 ab | 64.22 a | 66.76 |
| Kalınkara | 51.58 cd | ** | 71.73 ab | 20.58 b | 63.63 |
| Yassı Badem | 70.45 a | 70.45 a | 81.69 a | 19.71 b | 69.83 |
| Open pollination | 63.62 ab | 72.62 a | 36.90 bc | 80.42 a | 83.03 |

*The differences among the treatments indicated with the same letter vertically were not significant at P<0.05. **: Not determine

**Table 2**. Effect of pollen source on nut set of hazelnut cultivars in 2016 (%)

| Pollinizer Cultivars | Main Cultivars | | | | |
|---|---|---|---|---|---|
| | Tombul | Palaz | Çakıldak | Foşa | Allahverdi |
| Tombul | 35.60 d* | 50.56 ab | 67.32 bc | 55.54 b | 55.10 b |
| Palaz | 65.28 bc | 8.47 c | 86.82 a | 26.27 e | 67.02 ab |
| Çakıldak | 80.60 a | 60.52 a | 32.53 e | 51.11 b | 64.35 ab |
| Foşa | 39.27 d | 56.01 ab | 63.85 bc | 41.67 bcd | 74.88 a |
| Allahverdi | 75.66 ab | 42.20 b | 45.75 d | 56.60 b | 22.48 c |
| Sivri | 55.35 c | 39.60 b | 62.65 c | 30.04 de | 69.20 ab |
| Kalınkara | 60.52 c | 49.46 ab | 57.09 cd | 34.92 cde | 56.98 b |
| Yassı Badem | 56.79 c | 43.45 b | 76.73 b | 48.59 bc | 66.27 ab |
| Open pollination | 75.4 ab | 62.57 a | 53.48 cd | 76.41 a | 76.97 a |

*The differences among the treatments indicated with the same letter vertically were not significant at P<0.05.

In addition to self-pollination, Tombul, Çakıldak, Sivri, Kalınkara and Yassı Badem pollination applications were considered partially compatible in both years of the experiment in Foşa cultivar. On the other hand, Palaz and Allahverdi pollination applications, which were evaluated as absolute incompatibilities in 2015, were partially compatible in 2016 (Table 3-4).

In both years of the experiment, there was a partial compatible in all pollination applications of Allahverdi cultivar (Table 3-4).

Absolute incompatibilities in the self-pollination of Palaz cultivar in both years (Table 3-4).

Erdoğan et al. (2005), determined that Turkish hazelnut cultivars were able to obtain nut even self-pollination applications and therefore defined Turkish hazelnut varieties as 'partially compatible'. Erdoğan and Mehlenbacher (2000), as a result of hybridization in 8 hazelnut species determined between 0-78% of the nut set and hazelnut species with more than 10% of the nut set of completely compatible, between 5-10% partially compatible, less than 5% self-incompatibility.

When the effects of pollinizers on pollen compatibility and flowering times were evaluated together; it was defined that Allahverdi for Tombul, Foşa for Palaz, Tombul for Çakıldak, Çakıldak and Allahverdi for Foşa, and Foşa are the best pollinizer for Allahverdi.

**Disclosure statement**

No potential conflict of interest was reported by the author

**Table 3**. Index of pollen incompatibility (IPI) and incompatibilitiy situation (IS) in different of hazelnut cultivars crosses in 2015

| Pollinizer cultivars | Main cultivars | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tombul | | Palaz | | Çakıldak | | Foşa | | Allahverdi | |
| | IPI | IS | IPI | IS | IPI | IS | IPI | IS | IPI | IS |
| Tombul | 0.98 | partially compatible | 0.79 | partially compatible | 1.87 | completely compatible | 0.30 | partially compatible | 0.95 | partially compatible |
| Palaz | 0.73 | partially compatible | 0.10 | incompatible | 2.02 | completely compatible | 0.10 | incompatible | 0.77 | partially compatible |
| Çakıldak | 0.88 | partially compatible | 0.64 | partially compatible | 0.73 | partially compatible | 0.82 | partially compatible | 0.78 | partially compatible |
| Foşa | 1.10 | completely compatible | 0.85 | partially compatible | 1.72 | completely compatible | 0.82 | partially compatible | 0.81 | partially compatible |
| Allahverdi | 0.78 | partially compatible | 0.80 | partially compatible | 1.86 | completely compatible | 0.14 | incompatible | 0.77 | partially compatible |
| Sivri | 0.72 | partially compatible | 0.30 | partially compatible | 1.73 | completely compatible | 0.80 | partially compatible | 0.80 | partially compatible |
| Kalınkara | 0.81 | partially compatible | - | - | 1.94 | completely compatible | 0.26 | partially compatible | 0.77 | partially compatible |
| Yassı Badem | 1.11 | completely compatible | 0.97 | partially compatible | 2.21 | completely compatible | 0.25 | partially compatible | 0.84 | partially compatible |
| Open pollination | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | |

**Table 4**. Index of pollen incompatibility (IPI) and incompatibilitiy situation (IS) in different of hazelnut cultivars crosses in 2016

| Pollinizer cultivars | Main cultivars | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tombul | | Palaz | | Çakıldak | | Foşa | | Allahverdi | |
| | IPI | IS | IPI | IS | IPI | IS | IPI | IS | IPI | IS |
| Tombul | 0.47 | partially compatible | 0.81 | partially compatible | 1.26 | completely compatible | 0.73 | partially compatible | 0.72 | partially compatible |
| Palaz | 0.87 | partially compatible | 0.14 | incompatible | 1.62 | completely compatible | 0.34 | partially compatible | 0.87 | partially compatible |
| Çakıldak | 1.07 | completely compatible | 0.97 | partially compatible | 0.61 | partially compatible | 0.67 | partially compatible | 0.84 | partially compatible |
| Foşa | 0.52 | partially compatible | 0.90 | partially compatible | 1.19 | completely compatible | 0.55 | partially compatible | 0.97 | partially compatible |
| Allahverdi | 1.00 | completely compatible | 0.67 | partially compatible | 0.86 | partially compatible | 0.74 | partially compatible | 0.29 | partially compatible |
| Sivri | 0.73 | partially compatible | 0.63 | partially compatible | 1.17 | completely compatible | 0.39 | partially compatible | 0.90 | partially compatible |
| Kalınkara | 0.80 | partially compatible | 0.79 | partially compatible | 1.07 | completely compatible | 0.46 | partially compatible | 0.74 | partially compatible |
| Yassı Badem | 0.75 | partially compatible | 0.69 | partially compatible | 1.43 | completely compatible | 0.64 | partially compatible | 0.86 | partially compatible |
| Open pollination | 1.00 | | 1.00 | | 1.00 | | 1.00 | | 1.00 | |

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