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Incompatibility Alleles Expressed in Pollen of Turkish Hazelnut Cultivars

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Abstract: Sphorophytic pollen-stigma incompatibility is a characteristic of the genus *Corylus*. Incompatibility alleles expressed in the pollen of Turkish hazelnut cultivars were identified. Cultivars were selected from the field collection at Giresun, and were used as the pollen parents. Tester plants, whose S-alleles were known, were located in Ankara and Corvallis. Compatible crosses produced masses of long and parallel tubes while incompatible crosses produced very short tubes that often curved or ended with a pronounced bulb. The incompatibility alleles S_2, S_5, S_8, S_{10}, S_{12}, S_{21} and S_{24} were identified in the pollen of Turkish cultivars. Pollen of Palaz and Yuvarlak Badem expresses S_2, pollen of Foşa, Mincane and Sivri expresses S_8, pollen of Kan, Cavcava, Acı and Kargalak expresses S_{10}, pollen of İncekara, Kalınkara and Uzunmusa expresses S_{21}, pollen of Yassı Badem expresses S_5, pollen of Tombul expresses S_{12} and pollen of Çakıldak expresses S_{24}.

Key Words: *Corylus avellana*, hazelnut, pollen-stigma incompatibility, fluorescence microscope

Türk Fındık Çeşitlerinin Polenlerinde Bulunan Uyuşmazlık Allellerinin Belirlenmesi

Özet: Sporofitik polen–stigma uyuşmazlığı *Corylus* cinsinin karakteristik bir özelliğidir. Bu çalışmada, Türk fındık çeşitlerinin polenlerinde ekspres olan uyuşmazlık allelleri belirlenmiştir. Giresun' daki kolleksiyon bahçesinde bulunan çeşitler çiçek tozu kaynağı olarak, Ankara ve Corvallis' de bulunan ve S-allelleri bilinen çeşitler ve genotipler ise test bitkisi olarak kullanılmıştır. Uyuşur kombinasyonlarda çiçek tozları kütleler halinde, uzun ve birbirine paralel çim boruları oluşturmuştur. Uyuşmaz kombinasyonlarda ise çim borularının çok kısa kaldığı, genellikle kıvrık şekilde olduğu ya da ucunda şişkinlik meydana geldiği görülmüştür. Türk fındık çeşitlerinin çiçek tozlarında S_2, S_5, S_8, S_{10}, S_{12}, S_{21} ve S_{24} uyuşmazlık allellerinin bulunduğu tespit edilmiştir. Palaz ve Yuvarlak Badem çeşitlerinde S_2, Foşa, Mincane ve Sivri çeşitlerinde S_8, Kan, Cavcava, Acı ve Kargalak çeşitlerinde S_{10}, İncekara, Kalınkara ve Uzunmusa çeşitlerinde S_{21}, Yassı Badem çeşidinde S_5, Tombul çeşidinde S_{12} ve Çakıldak çeşidinde ise S_{24} allellerinin ekspres olduğu belirlenmiştir.

Anahtar Sözcükler: *Corylus avellana*, fındık, polen-stigma uyuşmazlığı, floresans mikroskobu

Introduction

Self-incompatibility is a specific mechanism that prevents self-fertilization and encourages fertilization by genetically unrelated individuals (1-4). The control of self-incompatibility is generally attributed to a single S-locus expressing multiple alleles (3,5). Incompatibility occurs if the alleles expressed in the pollen and pistil are identical. There are 2 basic types of self-incompatibility, gametophytic and sporophytic.

In sporophytic self-incompatibility systems, the incompatibility reaction occurs at the pollen-stigma interface in the very early stages of germination, and inhibition of self-pollen is very rapid (2,5-8). Incompatible pollen usually fails to germinate or pollen tubes are prevented from penetrating into the style. The genotype of the parent plant (sporophyte) that produces the pollen grain determines the phenotype of the pollen.

Incompatibility in cultivated hazelnuts (*Corylus avellana* L.) was first reported by Schuster (9) and Johansson (10). Hazelnuts express sporophytic self-incompatibility controlled by a single S-locus with multiple alleles (11,12). Currently 26 unique S-alleles are known to exist (13,14). In female flowers, stylar S-alleles are codominant and pollen alleles are either codominant or dominant. A linear dominance hierarchy consisting of 8 levels exists among S-alleles in the pollen (13,14). Pollen in other sporophytically incompatible families, Brassicaceae and Asteraceae, is tricellular whereas *C. avellana* pollen is bicellular (15). The stylar surface is covered with dry papillae which is a characteristic of the sporophytic self-incompatibility system (15,16). The site of the incompatibility reaction is the stigmatic surface of the pistil (16). Incompatible pollen may hydrate and germinate on the stylar surface as does compatible pollen. However, no tubes were observed to penetrate into the style. Reduced germination, and coiled and bulbous pollen tubes are characteristics of incompatible reactions in hazelnut (16,17). Other wild species in the genus *Corylus* also exhibit pollen stigma-incompatibility (18).

Rejection of incompatible pollen grains or tubes is based upon the interaction between the products of identical S-alleles carried in the pollen and the pistil (1). Heslop-Harrison et al. (15) reported that S-factors in *C. avellana* are held in the pollen wall, and it is possible that they form one component of the poral proteins that are of sporophytic origin (from the tapetum). The sporophytically derived pollen wall glycoproteins were shown to be responsible for the rejection response induced in the stigmatic papillae in Brassicaceae (19).

Turkey is the most important hazelnut producer in the world, producing about 70% of the world crop. The main cultivars are Tombul (33%), Çakıldak (13.5%), Mincane (12.1%), Palaz (10.9%), Karafındık (10.3%) and Foşa (7.2%) (20). Pollen stigma incompatibility is an important step in choosing pollinizers when establishing an orchard and selecting parental combinations in breeding. Pollination and fruit set studies (21,22) or microscopic investigations using fluorescence microscopy (23,24) have been performed to determine the best pollinizers for some cultivars, but there is no information about their incompatibility alleles. The objective of this study was to identify the incompatibility alleles expressed in the pollen of Turkish hazelnut cultivars.

Materials and Methods

Standard Turkish cultivars in the field collections of the Hazelnut Research Institute, Giresun, were selected for allele identification and were used as pollen parents (Table 1). Tester plants, whose S-alleles were known, were located in the field collection at the Ankara University, Faculty of Agriculture, Department of Horticulture in Ankara. Additional testers and seedlings with known S-alleles were in the collection of the Department of Horticulture at Oregon State University in Corvallis (Table 2).

Two to five branches of each tester tree were emasculated by clipping catkins, and were covered with Tyvek bags (1 x 0.5 m) in late December (25). This was done to isolate female inflorescence and prevent exposure to air-borne pollen. A second Tyvek bag was used to cover and protect the inner bag from damage by wind. Only female flowers from covered branches were used for incompatibility testing.

When catkins of Turkish cultivars had elongated and were about to shed, they were brought to the lab in the afternoon and laid on paper in a single layer where they were kept at room temperature (18-20 ^{o}C) overnight to allow the anthers to dehisce. The pollen was collected the

Table 1. Turkish hazelnut cultivars used as pollen parents and their major cultivation areas.

Cultivars	Pomological group	Main production area
Çakıldak	Round	Ordu
Cavcava	Round	Trabzon
Foşa	Round	Trabzon, Bolu
Kalınkara	Round	Giresun, Ordu
Kan	Round	Trabzon
Kargalak	Round	Trabzon
Mincane	Round	Trabzon
Palaz	Round	Ordu, Samsun
Tombul	Round	Giresun, Samsun
Uzunmusa	Round	Ordu
Acı	Pointed	Ordu
İncekara	Pointed	Giresun
Sivri	Pointed	Giresun, Trabzon
Yassı Badem*	Long	Adapazarı, İzmit
Yuvarlak Badem*	Long	Adapazarı, İzmit

* Generally sold in husks and consumed fresh

Table 2. Tester plants with known S-alleles used as female for identifying the incompatibility alleles in hazelnut pollen.

Alleles*	Tester	Alleles in Tester**	
1	Barcelona	1	2
1	Ennis	1	11
2	OSU 20.058	2	2
3	Willamette	1	3
3	Nonpareil	1	3
4	OSU 194.001	4	4
5	Badem	5	15
5	Halls Giant	2	5
6	Henneman #3	6	10
7	Tonda G. d. Langhe	2	7
7	OSU 278.095	4	7
8	San Giovanni	2	8
9	Segorbe	9	23
10	Imperial de Trebizonde	2	10
11	OSU 278.121	4	11
12	OSU 55.077	2	12
12	OSU 382.026	12	23
13	USOR 98-83	6	13
14	Gem	2	14
15	OSU 39.044	11	15
16	OSU 485.010	11	16
17	Mortarella	2	17
18	Neue Riesennuss	18	25
19	OSU 452.026	4	19
20	OSU 455.087	9	20
21	OSU 168.026	2	21
22	OSU 219.133	4	22
23	OSU 385.003	4	23
24	OSU 54.041	4	24
25	Ordu	4	25
26	OSU 447.015	26	26

* S_{13} from interspecific hybrid Chinese Trazel Gellatly #4 was not included in this study.

** Underlined number indicates allele expressed in pollen

next morning, placed in glass vials with cotton stoppers, and stored at –20 °C until used. Pollen was collected from the Turkish cultivars for the purpose of identifying the alleles expressed in the pollen.

Pistillate flowers were collected from the emasculated branches of tester plants and placed on moist filter paper in petri dishes when styles protruded 2-5 mm. The flowers were pollinated by dipping the styles into the appropriate vial of pollen, and leaving them on moist filter paper (Whatman no. 1) in covered petri dishes. They were left at room temperature (18-20 °C) for 16-20 h prior to staining. Two inflorescences, each consisting of 10 or more styles, were used for each pollen-tester combination. The stigmatic styles were extracted from the inflorescence and placed on a microscope slide, along with a few drops of Aniline Blue (0.1 g of Aniline Blue, 0.71 g of K_3PO_4, 100 ml of distilled water). They were squashed using plastic cover slips. The pollen tubes were immediately examined at 100X with a fluorescence microscope.

Compatible and incompatible reactions were clearly identified as described by Mehlenbacher (1997). If pollen is compatible on a tester genotype, then neither allele of the tester is present in the pollen. If pollen is compatible on all of the testers, then the allele expressed in the pollen is a new allele. If pollen is compatible on all but 1 tester genotype, then the alleles expressed in the pollen are either homozygous or 1 allele is dominant, but the second allele is unknown. If a cultivar exhibits codominance in the pollen, the reaction will be incompatible on female flowers of tester(s) that contain one or both alleles common to that pollen. The dominance hierarchy (dominancy or codominancy) among the alleles (14) makes it clear that the testing of some alleles is not necessary, as in many cases it is not possible for them to be expressed as a second allele in the pollen.

Results

Compatible and incompatible reactions of the testers to pollen from the Turkish cultivars are listed in Table 3. In compatible crosses, pollen germinated well and produced masses of long parallel tubes with strongly fluorescing callose plugs as reported earlier (26). Incompatible crosses had a lower pollen germination rate and produced very short tubes that often curved or ended in a pronounced bulb.

The incompatibility alleles S_2, S_5, S_8, S_{10}, S_{12}, S_{21} and S_{24} were identified in the pollen of Turkish cultivars. Pollen of Palaz and Yuvarlak Badem expresses S_2, pollen of Foşa, Mincane and Sivri expresses S_8, pollen of Kan, Cavcava, Acı and Kargalak expresses S_{10}, and pollen of İncekara, Kalınkara and Uzunmusa expresses S_{21}. Pollen of Yassı Badem expresses S_5, pollen of Tombul expresses

Table 3. Compatible and incompatible pollen reactions of Turkish hazelnut cultivars.

Cultivars	Incompatible on tester	Compatible on testers *
Palaz	2	1,3,4,5,6,7,8,9,10,11,12,14,15,17,19,20,21,22,23
Yuvarlak Badem	2	1,3,4,6,7,8,9,10,11,12,14,15,17,19,20,21,22
Yassı Badem	5	1,2,3,4,6,7,8,9,10,11,12,14,15,16,17,18,19,20,21,22,23,25
Foşa	8	1,2,3,4,5,6,7,9,10,11,12,14,15,16,17,18,19,20,21,22,23,24,25,26
Mincane	8	1,2,3,4,5,6,7,9,10,11,12,14,15,16,17,19,20,21,22,23,24,25
Sivri	8	1,2,3,4,5,6,7,9,10,11,12,14,15,16,17,18,19,20,21,22,23,24,25
Acı	10	1,2,3,4,5,6,7,8,9,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
Cavcava	10	1,2,3,4,5,6,7,8,9,11,12,14,15,16,17,19,20,21,22,23,25
Kan	10	1,2,3,4,5,6,7,8,9,11,12,14,15,16,17,18,19,20,21,22,23,25
Kargalak	10	1,2,3,4,5,6,7,8,9,11,12,14,15,16,17,18,19,20,21,22,23,24,25
Tombul	12	1,2,3,4,5,6,7,8,9,10,11,14,15,16,17,18,19,20,21,22,23,24,25,26
İncekara	21	1,2,3,4,5,6,7,8,9,10,11,12,14,15,16,17,18,19,20,22,23,24,25
Kalınkara	21	1,2,3,4,5,6,7,8,9,10,11,12,14,15,16,17,18,19,20,22,23,24,25
Uzunmusa	21	1,2,3,4,5,6,7,8,9,11,12,14,15,16,17,19,20,22,23,24,25
Çakıldak	24	1,2,4,5,6,7,8,9,10,11,12,14,15,16,19,20,21,22,23,25,26

* The dominance hierarchy (14) makes it clear that the testing of some alleles is not necessary, as in many cases it is not possible for them to be expressed as a second allele in the pollen.

S_{12} and pollen of Çakıldak expresses S_{24}. Based on the dominance hierarchy of S-alleles in hazelnut pollen (14), the 15 Turkish cultivars included in this study represent 3 different levels, with 5 of the identified alleles (S_5, S_{10}, S_{12}, S_{21} and S_{24}) in the same tier, meaning they are codominant.

Discussion

Okay and Ayfer (23) studied incompatibility using fluorescence microscopy to find the best pollinizer for Tombul. Pollen of Foşa, Mincane, Kalınkara, Palaz and Sivri germinated and pollen tubes were observed in the styles of Tombul female flowers. Our results are consistent with theirs, as we found that these pollinizers contain S-alleles different from those of Tombul. Researchers also indicated that although all of the tested cultivars were compatible, Mincane, Sivri and Foşa pollen resulted in a higher number of pollen tubes at the base of the Tombul styles than did Kalınkara and Palaz pollen. No explanation was offered for this, but it is interesting that pollen of the first 3 cultivars expresses a common S-allele (S_8), while pollen of Kalınkara expresses S_{21} and pollen of Palaz expresses S_2.

Hazelnuts are self-incompatible and when we selfed Tombul we observed incompatible reactions. However, Okay and Ayfer (23) and Beyhan and Odabaş (24) reported compatibility after an observation of self-pollen tubes reaching the base of styles. Other researchers have obtained nut clusters from self-pollinated Tombul. Arıkan (21) reported a 42% cluster set, Çakır and Genç (22) reported a 27.5% cluster set, and Mehlenbacher and Smith (27) reported a 44% cluster set. Fruit set after self-pollination has also been reported on other cultivars and selections such as Palaz (33.8%), Çakıldak (29.3%), Kalınkara (26.5%) and Sivri (22.3%) (22), Montebello (20%), OSU 41.134 (34.6%) and OSU 43.025 (28.3%) (27). It is clear that at least some degree of self-compatibility is present in some hazelnut cultivars. Mehlenbacher and Smith (27) called this event partial self-compatibility. It appears that stigmas fail to recognize incompatible pollen and inhibit pollen tube growth in incompatible crosses. However, factors such as temperature, humidity and CO_2 concentration, or bud pollinations (in *Brassica*) are known to affect the level of self-compatibility (3).

Cross-pollination is required for good nut set in commercial orchards. Since female flowers emerge and

are receptive over a period of several weeks, it is recommended that orchards include at least 2 pollinizers to supply sufficient amounts of viable and compatible pollen during this period. In addition, warm weather promotes dichogamy by accelerating pollen release more than style exsertion (16). Although some Turkish cultivars appear to be partially self-compatible, the use of compatible pollinizers is strongly recommended, because cross-pollination gives a much higher cluster set resulting in higher yields (20,22,27). Based on the incompatibility alleles shown in Table 3, all of the cultivars could be used as pollinizers for Tombul which is the main cultivar in Turkey. However, pomological groups of cultivars (Table 1) should be taken into account. The use of pollinizers with pointed or long nuts would require separation of their nuts from the round nuts of the main cultivar after harvest because the hazelnut industry and export market rely on round nuts.

Turkish hazelnut cultivars appear to be groups of clones with similar appearance, but which show minor differences. As a result, some clones within a group may have different S-alleles. We identified the dominant S-alleles expressed in pollen of specific clones of cultivars. Additional testing would reveal the extent of variation for S-alleles within these cultivar groups. Our results with pollen from cultivars in Turkish collections are also consistent with the alleles identified from previous testing of female flowers from trees in the OSU collection: Kalınkara S_4 S_{21}, Kargalak (syn. Imperiale de Trebizonde) S_2 S_{10}, Palaz S_2 S_4, Sivri (Ocak #5) S_8 S_{10}, and Tombul (syn. Extra Ghiaghli) S_4 S_{12}.

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