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A preliminary study on pollen compatibility of some hazelnut cultivars in Iran

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Abstract: Pollen incompatibility is a major problem among hazelnut cultivars and can result in considerable crop loss in hazelnut orchards. Thus, identifying the level of self-compatibility of a cultivar and compatibility between cultivars are crucial aspects to selecting the proper pollenizers. Controlled self- and cross-pollinations were carried out in three local and four imported hazelnut cultivars. Based on cluster set, partial self-compatibility was found in cultivars Pashmine and Shastak, and complete self-compatibility in 'Tabestane'. The best pollenizers for the cultivars Pashmine, Tabestane, Shastak, Barcelona, Segorbe, Daviana and Merveille were Segorbe, Barcelona, Segorbe, Shastak, Shastak, Pashmine and, Pashmine, respectively.

1. Introduction

Pollen incompatibility is a recognition mechanism that enables plants to prevent inbreeding. Many flowering plants have these systems and prevent self-fertilization and subsequently prevent inbreeding depression (Hiscock, 2002). Pollen compatibility is an essential factor in breeding programs of fruit trees and for selection of the best pollenizers in orchard establishment for all fruit and nut species, including the European hazelnut (*Corylus avellana* L.) (Mehlenbacher, 1997). Most hazelnut cultivars are self-incompatible; self- and cross-incompatibility in hazelnut cultivars are widespread phenomena which are of the sporophytic type (Germain, 1994). Dominance relations may lead to reciprocal differences in pollen incompatibility between cultivars, a result that makes pollinizer selection a complicated decision (Hampson *et al*., 1993). Cross-pollination is crucial for efficient nut set in hazelnuts. At least two different pollinizers are recommended for commercial yields to ensure sufficient amounts of viable, compatible pollen when needed, since flowers continue to emerge for several weeks (Hampson, *et al*. 1993). Therefore, in commercial orchards there should be several verified compatible cultivars to achieve appropriate nut production. To do so, it is suggested to have 6 to 15% of orchard trees as pollenizers (Mehlenbacher and Miller, 1988).

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Several studies have been carried out to determine the level of self- and cross-compatibility in different hazelnut cultivars from different geographical regions (Mehlenbacher and Smith, 1991; Mehlenbacher, 1997; Erdogan and Mehlenbacher, 2000; Mehlenbacher and Smith, 2006; Vicol, *et al*., 2009; Mehlenbacher, 2014). There is a serious need to examine self- and cross-compatibility of hazelnut cultivars in different climates and regions to determine the most compatible pollenizers for main hazelnut cultivars in the region. Accurate information on this aspect could also enhance breeding efficiency and contribute to knowledge about hazelnut pollen-stigma incompatibility as well as helping to improve further efforts to study interspecific and intraspecific crosses (Molnar, 2011).

Notwithstanding the importance of this requirement, there is little information on pollen compatibility of hazelnut cultivars grown in Iran. The present investigation was undertaken as a preliminary study to determine the level of self- and cross-compatibility of three Iranian and four imported hazelnut cultivars.

2. Materials and Methods

The study was carried out in winter 2010 at the Astara Hazelnut Research Station in Astara, province of Guilan, Iran. Three Iranian cultivars including Shastak, Pashmine, and Tabestane, and four imported cultivars Barcelona, Daviana, Segorbe, and Merville (= Merveille de Bollwiller) were selected. Three trees of each cultivar and some

shoots of the trees were selected: for each tree of Iranian cultivars six shoots were selected (for self-pollination, open-pollination and pollination with four imported cultivars); and also for each tree of imported cultivars four shoots were selected (for open pollination and pollination with three Iranian cultivars). The experimental design was completely randomized blocks with each tree considered as one block.

Based on the method described by Mehlenbacher (1997), after removing the catkins, the selected shoots were isolated with long paper bags. The selected shoots for open-pollination were left unbagged to receive airborne pollen. At the same time pollination and pollen receptivity period of all cultivars were monitored and recorded. Since the life of pollen and the receptivity of stigma are short and there is a gap between the periods of female and male flowers bloom, it is essential to know the duration of their activity for all cultivars in the region.

Pollen was collected in January, before the beginning of pollen shedding, and kept in vials at -18°C. Controlled pollinations were performed by hand when styles appeared and became receptive. The number of pollinated pistillate inflorescences was recorded after pollination. Produced nut clusters were picked and counted in early September. The percentage of nut cluster set was determined as the ratio of the produced nut clusters to the pollinated inflorescences.

To evaluate the level of self- and cross-incompatibility, the index of self-incompatibility (ISI) was applied (Zapata and Arroyo, 1978). ISI is the ratio of nut set after self- or cross-pollination to nut set after open-pollination, as a potential compatible cross. When the ratio is ≤ 0.2, the cross is incompatible, 0.2-1 is partially compatible, and ≥ 1 is completely compatible. Since this ratio was applied to evaluate both self- and cross-incompatibility, the index of pollen-incompatibility (IPI) was used instead of ISI, as suggested by Seifi *et al*. (2011). The obtained data were analyzed by analysis of variance (ANOVA) using SAS software version 9.1 for Windows (SAS Institute, 2001).

3. Results and Discussion

Pollination and pollen receptivity of all cultivars are illustrated in figure 1. Dichogamy was predominant in all cultivars. All seven cultivars were protanderous, which was expected according to the literature (Germain, 1994). The most distinct dichogamy was obvious in the cultivar Tabestane. Although there was complete self-compatibility through hand pollination in this cultivar, its pollen will not touch its stigma when such dichogamy exists. However, it is noticeable that dichogamy is influenced by climatic conditions and may differ from year to year. The shortest and longest period of pollen release were recorded in 'Shastak' (20 days) and 'Merville' (35 days), respectively. Further, the shortest and longest period of pollen receptivity by stigma of pistillate flowers were recorded in 'Shastak' (10 days) and 'Tabestane' (45 days), respectively (Fig. 1). The activity of male inflorescence of 'Merville' had an appropriate overlap with receptivity of the female inflorescence of all studied cultivars.

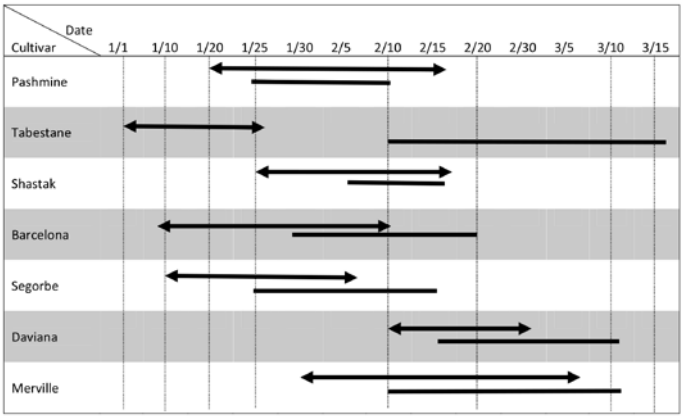

Fig. 1 - Period of bud break and female and male bloom of 10 hazelnut cultivars. ◄——► Male bloom; ▬▬ female bloom.

Pashmine

'Pashmine' is a local Iranian cultivar that is widespread throughout Iran. Little information is available about this cultivar's characteristics. Results showed that there was a significant difference between pollenizers in terms of nut set in 'Pashmine'. As can be seen in Table 1, there was no significant difference between self-pollination and open-pollination in 'Pashmine' which produced 39.4% nut set after self-pollination, indicating its partial self-compatibility. This result is roughly similar to nut set after self-pollination in the cultivar Tombul (44%) (Mehlenbacher and Smith, 1991). This level of self-compatibility is noticeable in this species and is reported here for the first time in Iran. The pollen of 'Barcelona', 'Daviana' and 'Merville' was completely incompatible with 'Pashmine' (IPI < 0.2) and produced no nuts. Pollen of 'Segorbe' was partially compatible with 'Pashmine' (IPI = 0.21) (Table 2).

Tabestane

This cultivar has a small nut and kernel and is widespread throughout traditional orchards in Iran. Statistical analysis revealed a highly significant difference between treatments (pollenizers) ($P < 0.01$) (Table 1). There was no significant difference between self- and open-pollination, and self-pollination resulted in 59.82% nut set, indcating complete compatibility (IPI = 1.22) (Table 2). This is a rare and interesting phenomenon in hazelnut. This level of self-compatibility was higher than the two other local pollenizers 'Pashmine' (39.4%) and 'Shastak' (19.33%). Nevertheless, this nut set percentage was lower than that after self-pollination in some genotypes reported by Mehlenbacher and Smith (2006).

Shastak

The size of nut and kernel of this local cultivar is medium. According to analysis of variance, pollenizers had a significant difference in terms of nut set. The highest nut set was obtained through open pollination (63.14%). 'Shastak' showed partial self-compatibility (IPI = 0.31) (Table 2). 'Barcelona', 'Daviana' and 'Merville' with IPI lower than 0.2 were considered as completely incompatible pollenizers (Table 2).

Barcelona

In the literature, the pollenizers 'Casina', 'Daviana' and 'Merville' have been suggested for 'Barcelona' (Wilkinson, 2005). Pollenizers for this cultivar were significantly different. Open pollination resulted in 50% nut set which was statistically different from other pollenizers ($P < 0.01$) (Table 1). Pollen of 'Pashmine' was completely incompatible with 'Barcelona' (IPI = 0) and produced no nut and consequently is not suggested for 'Barcelona'. More research is needed to ensure this finding.

Segorbe

This cultivar originated from France and has upright growth with medium to large nuts. Its pollen grain is shed in early winter and pistillate flowers are observable in mid-winter (Wilkinson, 2005). Observations demonstrated significant differences between treatments. The highest nut set in 'Segorbe' was obtained after open pollination (82.62%). This could be as a result of the effect of mixing various cultivars' pollen grains from more than 40 genotypes at the station. Based on calculated IPI, 'Tabestane' (0.22) and 'Shastak' (0.24) were partially compatible with 'Segorbe'; but 'Pashmine' was incompatible (Table 2).

Daviana

'Daviana' originated from England and is an upright growing cultivar with few root suckers and medium and oblong nuts. Its pollen grain is shed in mid-winter and pistillate flowers are observed in late winter. This cultivar is considered an appropriate pollenizer for 'Barcelona' and 'Butler' (Wilkinson, 2005). Based on analysis of variance, there was a significant difference between all pollenizers. The highest nut set resulted from open pollination (26.78%) which actually was not that much in such an orchard containing almost 40 different cultivars. 'Pashmine' was partially compatible (IPI = 0.35); however, the amount of nut set was low (Tables 1, 2). The pollenizers 'Tabestane' and 'Shastak' showed complete incompatibility. Therefore, according to the preliminary findings, the three mentioned pollen sources are not suggested for 'Daviana'.

Table 1 - Cluster set in controlled self- and cross-pollinations of some hazelnut cultivars (%)

Pollenizer \ Main cultivar	Pashmine	Tabestane	Shastak	Barcelona	Segorbe	Daviana	Merville
Pashmine	39.4±20.8 ab	-	-	0.00±0.00 c	2.38±2.38 b	9.48±1.97 a	25.16±3.41 b
Tabestane	-	59.82±15.61 a	-	17.26±11.28 b	18.35±6.33 b	1.59±1.59 b	18.38±6.62 b
Shastak	-	-	19.33±8.51 bc	17.76±5.88 b	19.44±15.47 b	1.19±1.19 b	20.73±1.49 b
Barcelona	8.4±4.55 bc	13.63±9.77 b	9.42±8.14 c	-	-	-	-
Segorbe	12.3±6.32 bc	10±3.71 b	30.47±4.38 b	-	-	-	-
Daviana	7.5±1.5 bc	6.72±4.15 b	5.84±2.95 c	-	-	-	-
Merville	5.61±1.98 c	5.75±3.02 b	2.3±1.15 c	-	-	-	-
Open	57.38±6.81 a	49.02±8.22 a	63.14±6.31 a	50±1.92 a	82.62±7.98 a	26.78±9.71 a	76.18±6.17 a

Mean±SE. Means within a column followed by the same letter are not significantly different ($P<0.01$).
Within the table, hyphen (-) means that the cluster set was not determined where it was not aimed.

Table 2 - Index of pollen incompatibility (IPI) in different crosses of hazelnut cultivars

Pollenizer \ Main cultivar	Pashmine	Tabestane	Shastak	Barcelona	Segorbe	Daviana	Merville
Pashmine	0.69	-	-	0.00	0.03	0.35	0.33
Tabestane	-	1.22	-	0.35	0.22	0.059	0.24
Shastak	-	-	0.31	0.36	0.24	0.044	0.27
Barcelona	0.15	0.28	0.15	-	-	-	-
Segorbe	0.21	0.20	0.48	-	-	-	-
Daviana	0.13	0.14	0.09	-	-	-	-
Merville	0.0017	0.12	0.04	-	-	-	-
Open	-	-	-	-	-	-	-

Within the table, hyphen (-) means that the cluster set was not determined where it was not aimed.

Merville

This cultivar is known as Hall's Giant in the United States and Australia and has been created from a seedling selection in Germany in 1788 (USDA, 2010). Its pollen is shed in late winter, after other cultivars, for a short time. Its pistillate flowers also appear in late winter (Wilkinson, 2005). Results showed that open pollination led to the highest nut set (76.18%) which was significantly different from the other pollenizers. 'Pashmine', 'Tabestane' and 'Shastak' showed partial compatibility with 'Merville', without any significant difference between them (Table 1).

4. Conclusions

In conclusion, based on cluster set observations, partial self-compatibility was found in the cultivars Pashmine and Shastak, and interestingly complete self-compatibility in Tabestane. The best pollenizers for the cultivars Pashmine, Tabestane, Shastak, Barcelona, Segorbe, Daviana and Merveille were Segorbe, Barcelona, Segorbe, Shastak, Shastak, Pashmine, and Pashmine, respectively. Since the observed compatibility in hazelnut cultivars may not be consistent over the years, complementary pollination studies should be carried out to verify the previous compatibility results and therefore document more reliable data.

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