

Studies on Self-Incompatibility Alleles in Some Progenies of Hazelnut (*Corylus avellana* L.) using Fluorescence Microscope

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ABSTRACT

This research is part of a programme for hazelnut breeding by controlled crosses. Incompatibility in hazelnut (*Corylus avellana* L.) is of the sporophytic type and controlled by single locus with multiple alleles. Fluorescence microscopy was used to study incompatibility in some progenies of Tonda Gentile delle Langhe (TGL) x Cosford. S-alleles were classified on the basis of growth or no growth of pollen tubes in styles after hand pollination in the laboratory with pollen testers, whose S-alleles are known. Observations were performed 20-24 h after pollination with fluorescence microscopy. Pollen-stigma compatibility relationships were determined in 34 progenies of TGL x Cosford and 4 pollen testers.

Key Words: *Corylus*; Hazelnut; Self-Incompatibility; S-alleles; Progenies

INTRODUCTION

Hazelnut (*Corylus avellana* L.) is at present an expanding crop in several countries due to increased demand by the processing industry. The European hazelnut (*C. avellana* L.) is monoecious, dichogamous and wind pollinated (Lagerstedt, 1975; Thompson, 1979a).

Staminate flowers (male flowers) are born in catkins at nodes on 1-year wood. Female flowers are formed singly at nodes on 1-year wood, in groups of 1-6 on catkin peduncles and on very short spurs on older wood, the proportion at each site varying according to the cultivar. The pistillate inflorescence consists of a tight cluster of 4-14 very small flowers, which are born in three locations within the overlapping bud scales at apex of a compound bud (Thompson *et al.*, 1996). Each female flower is formed by two styles. Fertilization of the ovules occurs at the end of May to beginning of June (Me *et al.*, 2000).

It is known that there is self and cross incompatibility in hazelnut. Pollen-stigma incompatibility in this plant is an important consideration in planning crosses in a breeding program and pollinizers for orchard planting. Therefore, it is necessary to know about the incompatibility between the main and the pollinizer cultivars in the orchards (Thompson, 1979; Mehlenbacher & Smith, 1991).

Self-incompatibility is classified as Gametophytic Self-Incompatibility (GSI) or Sporophytic Self-Incompatibility (SSI) based on whether the pollen behavior is determined by its own haploid genotype (GSI) or by the diploid genotype (SSI) of its parent (Matton *et al.*, 1994; Franklin *et al.*, 1995). Incompatibility in hazelnut (*C. avellana* L.) is of sporophytic type and under the control of a single locus with multiple alleles (Thompson, 1979a). The stigmatic surface is the site of the incompatibility reaction and pollen tube

growth is arrested in the stigmatic surface (Mehlenbacher *et al.*, 1988; Hampson *et al.*, 1993). In sporophytic incompatibility system, the pollen exine carries two S-alleles determined by plant which produced the pollen (Me *et al.*, 2000). In other words, pollen rejection in sporophytic system is controlled by the interaction of the self-incompatibility genotype of the pistil with the genotype of the pollen parent, and not with the haploid genotype of the pollen. Thus, each pollen grain in plants with sporophytic self-incompatibility presents the products of two S-alleles, and rejection occurs when either one of these alleles matches either of the S-alleles expressed in the pistil (Matton *et al.*, 1994).

Molecular studies of sporophytic self-incompatibility (SSI) have been carried out exclusively in *Brassica*. The self-incompatibility response occurs in the stigmatic papillar cells that carry on their surface two S-locus encoded proteins: the plasma membrane-anchored S receptor kinase (SRK) gene (Stein *et al.*, 1991) and the cell wall localized S-locus glycoprotein (SLG) (Nasrallah *et al.*, 1985). Both genes are highly polymorphic and SLG shares a high degree of sequence identity with the extracellular domain of SRK. (Takasaki *et al.*, 2000; Silva *et al.*, 2001) conclusively showed that SRK is the primary determinant of self-incompatibility in the pistil, and that SLG acts to promote the full manifestation of the SI response through an unknown mechanism (Takayama & Isogai, 2003).

The physiology of the self incompatibility reactions of all the SSI species so far examined (*Brassica* & *Ipomoea*) appears to be similar. The stigma surface is dry and the pollen trinucleate. Self pollination is generally inhibited early, either before or just after germination (Franklin *et al.*, 1995). However, Me *et al.* (1983, 2000) and Heslop-Harrison *et al.* (1986) found binucleate pollen in hazelnut.

To date, 28 S-alleles (Mehlenbacher, 2001).
 S-alleles but always co-hierarchy of S-alleles (Mehlenbacher, 2001).
 about compatibilities of cultivars and pomology (Me *et al.*, 1983, 1993, Pomper *et al.*, 2001).
 The aim of this study was to construct a field interplanting program

Table II. Compatibility relationships among 4 testers and 34 progenies from TGL x Cosford

Cultivars and Progenies	Genotype of Pistillate	Genotype of pollen							
		1-3-12-18-38-49-50-60-65-87-89.	5-20-33-36-44-67.	8-19-21-35-40-57-58-64.	10-15-25-26-32-37-48-66-69.	TGL	Cosford	Tonda di Giffoni	OSU 278-121
1-3-12-18-38-49-50-60-65-87-89.	S2 S3	-	-	-	+	+	-	-	+
5-20-33-36-44-67	S3 S7	-	-	+	-	-	-	+	+
8-19-21-35-40-57-58-64	S2 S11	+	+	-	+	+	+	-	-
10-15-25-26-32-37-48-66-69	S7 S11	+	+	+	-	-	+	+	-
TGL	S2 S7	+	+	-	-	-	+	-	+
Cosford	S3 S11	-	-	+	+	+	-	+	-
Tonda di Giffoni	S2 S23	+	+	-	+	+	+	-	+
OSU 278-121	S4 S11	+	+	+	+	+	+	+	-

+ = Compatible - = Incompatible S? = S- allele (underlined) expressed in pollen

MATERIALS AND METHODS

The seedling trees in this study resulted from controlled crosses in Piemonte region, made as part of the breeding programs at the Department of Arboriculture and Pomology of Torino University (Italy). Thirty eight seedling trees were obtained from crosses between Tonda Gentile delle Langhe (TGL) as female parent and Cosford as male parent. In each seedling tree two branches were marked for emasculation and catkins clipped in mid-December. Emasculated branches were bagged with two layers of bags, to protect female inflorescences from wind-borne pollen. When staminate catkins elongated and were about to shed pollen, they were collected, placed on sheet of paper in the laboratory, and allowed to dry overnight at room temperature (18-20°C). The following day, the catkins were discarded then pollen collected and stored in cotton-stoppered vials in freezer (-20°C). Pollen collected from four testers (TGL, Cosford, Tonda di Giffoni and OSU278-121), whose alleles are known (Table I).

Female clusters were detached from bagged limbs, when styles protruded 2-6 mm and placed in Petri dishes on a double layer of moist filter paper. Pollinations were made in the laboratory on the day of collection, by both, using brush or dipping the stigmatic styles in the pollen vial, shaking off excess pollen and leaving the flowers on double

Table I. Pollen testers for incompatibility alleles and S-alleles combinations in progenies of TGL x Cosford

Testers	Genotype of Pollen testers	Genotype of pistillate in progenies			
		S2S3	S2S11	S3S7	S7S11
TGL	S2 S7	+	+	-	-
Cosford	S3 S11	-	+	-	+
OSU278-121	S4 S11	+	-	+	-
T.Giffoni	S2 S23	-	-	+	+

(+) = Compatible; (-) = Incompatible; S? =_S-allele (underlined) expressed in pollen

penetrate the stigmatic surface. Many of these tubes terminated in a pronounced bulb, as described previously by Mehlenbacher (1997a), and Erdogan and Mehlenbacher (2001).

The identification of S-alleles in selections was performed as follows: the crosses between TGL (S2S7) and Cosford (S3S11) has four possible S-alleles combinations: S2S3, S2S11, S3S7 and S7S11. Pollen from four testers were chosen, each expressing one of the four alleles S2, S3, S7 and S11. Stigmas of the seedlings of this progeny were hand pollinated with these pollen. The incompatibility reaction to a given tester pollen demonstrated the presence of the same S-allele also in the stigma of the seedling.

Cross TGL (S2S7) X Cosford (S3S11). Possible S-alleles combinations in the progeny: S2S3, S2S11, S3S7, S7S11. For finding the S-alleles in one seedling (S?S?)

Pollination of the seedling with TGL (expressed S7)

Incompatible cross----- S7S?

Pollination of the seedling with Cosford (expressed S3).

Compatible cross----- S7S?, this showed that seedlings had not have S3-allele

Pollination of the seedling with T. di.Giffoni (expressed S2).

Compatible cross -----S7S?, this showed that seedlings had not have S2-allele

Pollination of the seedling with OSU278-121 (expressed S11).

Incompatible cross-----S7S11

Other progeny identified by this method is reported in Table I and II. However, in a few progenies determined only one allele. Mutual compatibility exists between TGL and progenies that whose alleles are known (S2S3). Progenies with S3S7 alleles fertilizes TGL, but can't be fertilized by it. Also mutual compatibility exists between Cosford cultivar and progenies with S2S11 and S7S11 alleles. With regard to agricultural aspects it is follows that choice of pollinizers to be consociated in specialized orchards is restricted to only combinations with mutual interfertility as it's obviously necessary that the pollinizers be also fertilized and bear fruit.

The quality of the female inflorescence was importance in obtaining very clear and easily distinguishable reaction. Fresh female flowers should be used whenever

possible, and responded very clearly. Flowers quality declines when they held for longer periods. Experience has also shown that females of some progenies typically give poor reactions while those of others give excellent reactions, as described previously by Mehlenbacher (1997a, b), and Bassil and Azarenko (2001). Mehlenbacher (1997a) reported that S11 allele is unusual in pollen, tends to germinate fairly well and several long tubes are present in incompatible reaction, making it especially difficult to determine if a pollination is compatible or incompatible. Also, our experiments showed similar results.

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