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# Physiological and genetic determination of self-compatibility in an almond breeding progeny

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**SUMMARY –** Traditionally, self-compatibility has been assessed in almond by microscopic observation of the pollen tube growth from the stigma to the style base after self-pollination in laboratory conditions. Recently, *S*-alleles have been determined through the expression of the product of each allele in the style, the stylar *S*-RNases. Previous studies have shown the presence of self-incompatible seedlings in progenies of 'Ferralise' ($S_1S_3$) x 'Tuono' ($S_1S_f$), whereas in other crosses from parents with the same *S* genotypes, like 'Ferragnès' x 'Tuono', all seedlings were found to be self-compatible, thus raising some doubts on the form of self-compatibility transmission. In our work, we have studied a 'Ferralise' x 'Tuono' progeny of 91 seedlings. The stylar *S*-RNase determination was made in 87 seedlings and all were characterized as self-compatible because they possessed the $S_f$ allele. However, after four years of microscopic observations we have concluded that 10 seedlings are self-incompatible because their own pollen is unable to reach the base of the style, whereas 8 seedlings show inconclusive behaviour. Consequently, other genetic factors, besides the presence of the $S_f$ allele, may be responsible for the pollen compatibility reaction in this progeny. One of these factors may be inbreeding, as shown by the presence of some dwarf seedlings in this inbred progeny. The expression of these genes in some individuals of the 'Ferralise' x 'Tuono' progeny studied would explain the different results obtained in comparison with the 'Ferragnès' x 'Tuono' progeny.

**Key words:** *P. amygdalus*, self-compatibility transmission, *S*-Rnases, pollen tube growth, inbreeding.

***RESUME –*** *"Détermination physiologique et génétique de l'autocompatibilité dans les descendances d'amandiers en amélioration". Traditionnellement, l'auto-compatibilité a été étudiée chez l'amandier par observation microscopique de la croissance des tubes polliniques depuis le stigmate jusqu'à la base du style après l'auto-pollinisation en conditions de laboratoire. Tout récemment, les allèles* S *ont été déterminés par l'expression du produit de chaque allèle dans le style, les* S*-Rnases stylaires. Des études antérieures ont montré la présence de semis auto-incompatibles dans les descendances de 'Ferralise' (*$S_1S_3$*) x 'Tuono' (*$S_1S_f$*), tandis que dans des croisements de parents avec les mêmes génotypes* S*, comme 'Ferragnès' x 'Tuono', tous les semis ont été auto-compatibles, ce qui soulève quelques doutes sur la voie de transmission de l'auto-compatibilité. Dans ce travail on a étudié une famille de 84 semis de 'Ferralise' x 'Tuono'. Pour la détermination de la* S*-RNase stylaire, les 84 semis ont tous été caractérisés comme auto-compatibles parce que possédant l'allèle* $S_f$*. Pourtant, après quatre années d'observations microscopiques, on a conclu que 9 semis sont auto-incompatibles parce que le propre pollen n'est pas capable d'atteindre la base du style, tandis que 8 semis ont montré un comportement peu concluant. Par conséquent, d'autres facteurs génétiques, en plus de la présence de l'allèle* $S_f$*, doivent être responsables de la réaction de compatibilité du pollen dans cette famille. Un de ces facteurs peut être la consanguinité, comme le montre la présence de quelques semis nains dans cette famille consanguine. L'expression de ces gènes chez quelques individus de la famille 'Ferralise' x 'Tuono' étudiée pourrait expliquer les différents résultats obtenus en comparaison avec la famille 'Ferragnès' x 'Tuono'.*

***Mots-clés:*** P. amygdalus*, transmission de l'auto-compatibilité,* S*-Rnases, croissance des tubes polliniques, consanguinité.*

## Introduction

Almond *(P. amygdalus* Batsch) shows a gametophytic self-incompatibility system (Socias i Company, 1990) controlled by a multiallelic locus, known as locus S (Gagnard, 1954). Self-compatibility has become a priority in the almond breeding programmes and the search for new cultivars is focused on the evaluation of desirable traits in autogamous seedlings (Socias i Company and Felipe, 1988). 'Tuono', a self-compatible cultivar from the Italian region of Puglia has been used in many breeding programmes as a source of self-compatibility for the new cultivars (Socias i Company, 1990; 2002). Self-compatibility has been traditionally assessed in the segregant families by the microscopic observation of the own pollen tubes reaching the style base during at least two years (Socias i Company and Felipe, 1988).

Each *S* allele codifies for a specific *S*-Rnase which is secreted in the extracellular matrix of the style transmitting tissue (Mascarenhas, 1993), probably degrading the RNA of the incompatible pollen tubes, stopping their growth (McClure *et al*., 1990). Recently, the "non-equilibrium pH gradient electrophoresis" (NEpHGE) has been applied to the identification of the stylar *S*-RNases and to the correlation of these proteins with the almond self-incompatibility alleles (Batlle *et al*., 1997, Bošković *et al*., 1998; 1999).

Although self-compatibility transmission in almond was clearly explained (Grasselly *et al*., 1981, Socias i Company, 1984, Dicenta and García, 1993), Grasselly (1985), after bagging branches in an offspring 'Ferralise' ($S_1S_3$) x 'Tuono' ($S_1S_f$), found that only 2/3 of the population was self-compatible, when, after the genotypes of the parents and their ancestry (Fig. 1), all the seedlings were expected to be self-compatible. Duval *et al*. (2001) found that self-compatibility transmission was complete in this family after examining their stylar *S*-Rnases, but was not horticulturally confirmed. Our objective was to study the self-compatibility transmission in a family 'Ferralise' x 'Tuono' from two points of view in order to assess if self-incompatible seedlings may appear in this offspring even if possessing the $S_f$ self-compatibility allele.

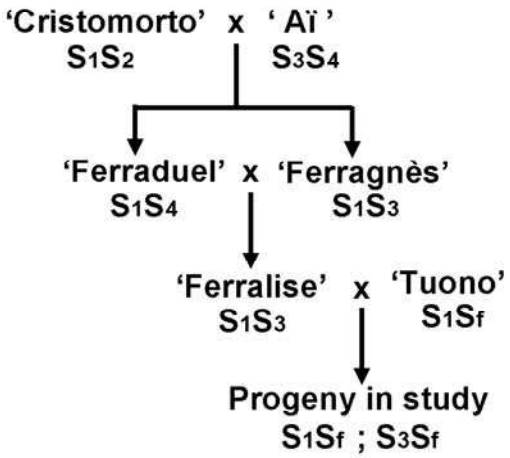


Fig. 1. *S* genotypes of the parents involved in the progeny studied.

## Materials and methods

A family of 91 seedlings from the cross 'Ferralise' ($S_1S_3$) x 'Tuono' ($S_1S_f$), grown at the SIA fields was used for this study. During blooming of the years 1999-2002, 20 bud flowers at stage D (Felipe, 1977) were collected from each seedling, emasculated in the laboratory and placed in a tray with a semi-rigid plastic mesh allowing the contact of the flower peduncles with the tray water (Fig.2). The anthers from the same 20 flowers were separated and allowed to dry for 48 hours and their pollen was used to hand pollinate the pistils of the same genotype in the tray. Pistils were collected 96 hours after pollination and autoclaved for 20 minutes at 1.2 kg/cm$^2$ in a 5% solution of sodium sulphite. Samples were maintained at 4ºC until observation.

Pistils were prepared for microscopic observation of pollen tube growth according to Linskens and Esser (1957), modified by Socias i Company (1979). Twelve pistils were examined per genotype, observing the level of pollen tube progression in each style. A genotype was considered self-compatible when pollen tubes reached the base of at least 8 of the 12 styles examined during a minimum of two years.

The identification of the self-incompatibility alleles was undertaken by separation of the stylar *S*-RNases by the NEpHGE procedure. During blooming of the years 2001 and 2002, 30 pistils were collected for each genotype from bud flowers at stage D and kept at –80ºC until utilization. The procedure of the protein extraction from the styles, the electrophoresis and the staining of the acrylamide gels followed the method of Bošković *et al*. (1997).

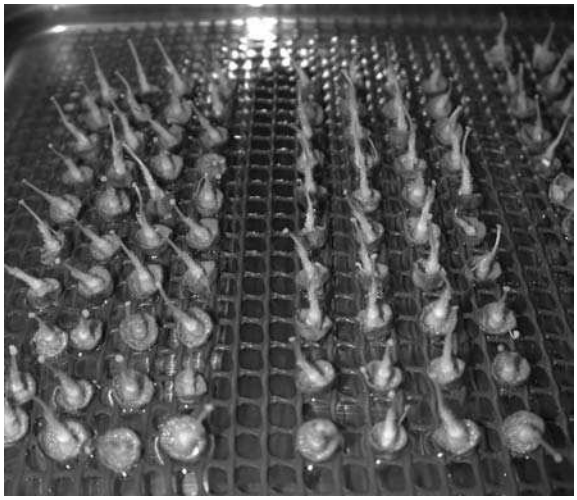

Fig. 2. Emasculated flowers placed in a tray with water in the laboratory.

## Results

The examination of the pollen tube growth showed that out of the 91 genotypes of the cross 'Ferralise' x 'Tuono', 70 (77%) showed a self-compatible behaviour, 10 (11%) a self-incompatible behaviour, and eight were doubtful. Although the seedlings were eight years old, three seedlings did not produce enough flowers for the study (Table 1).

Table 1. Distribution of phenotypes and genotypes

| *S*-Rnase genotype | Pollen tube growth phenotype | | | | Total |
|---|---|---|---|---|---|
| | Self-compatible | Doubtful | Self-incompatible | Unknown | |
| $S_1S_f$ | 36 (39,5%) | 4 (4,3%) | 4 (4,3%) | 2 (2,2%) | 46 (50,5%) |
| $S_3S_f$ | 31 (34,1%) | 4 (4,3%) | 5 (5,5%) | 1 (1,1%) | 41 (45,0%) |
| Unknown | 3 (3,3%) | 0 (0,0%) | 1 (1,1%) | 0 (0,0%) | 4 (4,3%) |
| Total | 70 (76,9%) | 8 (8,8%) | 10 (10,1%) | 3 (3,3%) | 91(100,0%) |

Only 88 genotypes from the total of 91 could be studied for their *S*-RNase alleles. In 46 of them, only the *S*-RNase of the $S_1$ allele was identified, thus assigning the genotype $S_1S_f$ to them, as the allele $S_f$ does not show any band. In 41 genotypes, only the $S_3$ allele was identified, thus assigning the genotype $S_3S_f$ to them for the same reason (Table 1). In a single seedling two bands were detected, one identified as $S_3$, but the other was not identified, thus assigning the genotype $S_3S_?$ to this seedling. The distribution of the maternal alleles followed a 1:1 ratio ($X^2$=0.29). The bands obtained through the electrophoresis of the stylar *S*-RNases, the gel interpretation and *S*-genotypes deduced from them are shown in Fig. 3.

## Discussion

The cross 'Ferralise' x 'Tuono' would produce a fully self-compatible offspring because when 'Ferralise' ($S_1S_3$) is pollinated by 'Tuono' pollen ($S_1$ and $S_f$), only the 'Tuono' pollen carrying the $S_f$ allele would be able to fertilize the 'Ferralise' ovules, consequently producing only two genotypes in the offspring, $S_1S_f$ and $S_3S_f$. The analysis of the *S*-RNases of 87 seedlings from this cross has confirmed this hypothesis and the genotype segregation has been, as expected, 1:1 (46 $S_1S_f$ : 41 $S_3S_f$) and agrees with the previous results of Duval *et al*. (2001). Thus all 87 seedlings would be self-compatible because they carry the $S_f$ allele. However, the results of the pollen tube growth showed the presence of self-incompatible and doubtful seedlings (Table 1). Grasselly (1985) had already

found self-incompatible seedlings in a family obtained from the same parents as opposed to the absence in the progeny from the cross 'Ferragnès' x 'Tuono', which involve the same *S* alleles.

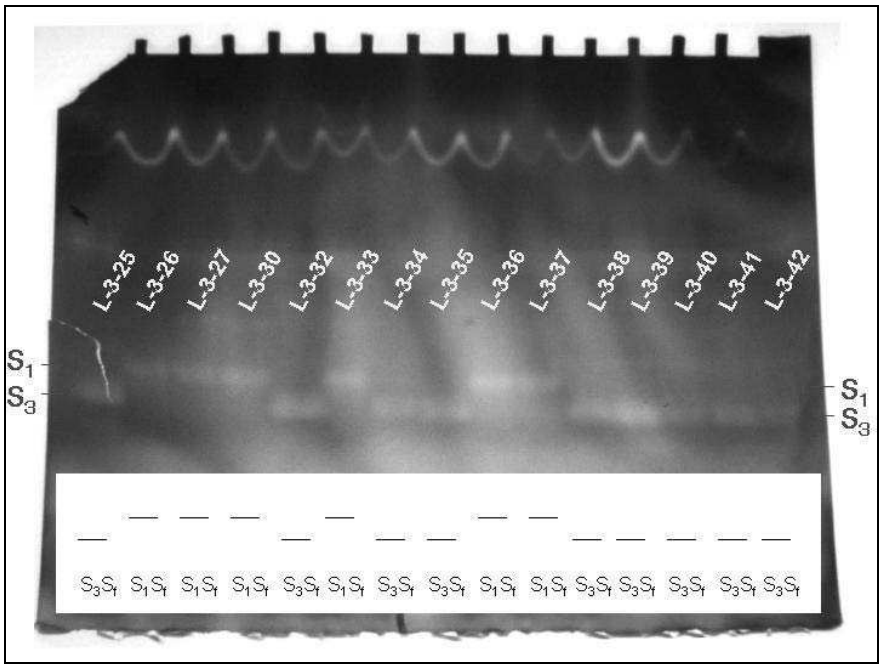


Fig. 3. Polyacrylamide gel interpretation and *S*-genotypes deduced.

The NEpHGE technique assumes that the absence of one of the two bands of the S-RNases implies the presence of the $S_f$ allele, but this might not be the case. As an example, there is no evidence that in the cross 'Ferralise' ($S_1S_3$) x 'Tuono' ($S_1S_f$) there is no possibility for an ovule $S_1$ of 'Ferralise' to be fertilized with an $S_1$ pollen from 'Tuono', after overcoming the self-compatibility barriers. In this hypothetical case, a self-incompatible homozygous $S_1S_1$ seedling would be produced, showing a single band of stylar *S*-RNases, corresponding to the $S_1$ allele and would be considered as self-compatible and carrying the $S_f$ allele with the genotype $S_1S_f$.

Another aspect regarding the NEpHGE technique is that it characterises the *S* locus in the style, the female part of the flower, obviating the expression of the *S* locus in the pollen, the male flower part. Pollen tube growth evaluate the interaction of the locus *S* expression both in the style and the pollen, and, thus, the joint behaviour of the gametophytic self-incompatibility system. Consequently, the NEpHGE technique gives information on the genotype of the plant through its female expression, but not on the gene behaviour.

On the contrary, through pollen tube growth it is possible to obtain information on the joint behaviour of the self-incompatibility gene. If most pistils show the arrival of pollen tubes at the style base, the seedling may be concluded to be self-compatible. The higher the number of pollen tubes reaching the style base, the higher the possibility of ovule fertilization. As a consequence, the proportion of styles with pollen tubes at their base and the number of pollen tubes reaching this base give real horticultural information on the ability of self-fertilization of a seedling.

Furthermore, in the genealogy of the cross 'Ferralise' x 'Tuono' there are some consanguinity relations (Fig. 1) because 'Ferralise' comes from the cross of two full siblings, 'Ferragnès' and 'Ferraduel', and their parent 'Cristomorto' is from the Italian region of Puglia, as well as 'Tuono'. This consanguinity may affect the expression of deletereous genes for the reproductive biology of some seedlings, as it also affects the vigour, as shown by the presence of dwarf seedlings and others producing a reduced number of flowers.

## Conclusions

The presence of the $S_f$ allele not always ensures the expression of self-compatibility, as shown in some seedlings from the family 'Ferralise' x 'Tuono'. This implies that the identification of the *S* alleles by separation of the stylar *S*-RNases by NEpHGE is useful to identify the self-incompatible seedlings in a segregant population of a breeding programme and to discard them. In the other seedlings, self-compatibility will need to be evaluated from a horticultural point of view, by their pollen tube growth.

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