A genetic approach to the transmission of self compatibility in almond (Prunus amygdalus Batsch)

R. Socias i Company
Departamento de Fruticultura
INIA - CRIDA-O3
Apartado 727
Zaragoza, Spain

INTRODUCTION

Almond breeding programs have stressed the development of self compatible cultivars since its importance has been carefully considered (Socias i Company, 1978). The present self-compatible cultivars and forms have been the initial source from which the programs have developed, as there has been a search for selections covering a broader range of requirements than the existing forms. However, whereas the transmission of self-compatibility to the offsprings has been confirmed (Socias i Company and Felipe, 1977), its mechanism has not been deeply studied, and it would be very useful in the programming of breeding.

Almond is a species with a single-locus gametophytic type of self-incompatibility, found in the Rosaceae family (Crowe, 1964), as suggested by the behaviour of its pollination and pollen tube growth (Socias i Company et al., 1976) and studies of related species of the genus *Prunus,* such as cherry (Crane and Brown, 1937). This type is ruled by a series of alleles at a single locus, the S alleles. The incompatibility reaction is determined by the genotypes of the pistil and of the pollen grain, whose tube grows slowly or is inhibited in pistils bearing the same allele. If an almond cultivar, a diploid plant, has the S_1S_2 genotype, it produces two different kinds of pollen grains, those of S_1 and S_2 genotypes. Both are unable to grow in the pistils of the same plant because the pistils carry the same alleles as the grain since those are diploid. This interpretation was proposed by East and Mangelsdorf (1925) and explains the cross-compatibility of a plant with its progeny, as the progeny of a S_1S_2 plant must be S_1S_i or S_2S_i, i $\neq$ 1, 2, and the existence of cross-compatible groups as has been shown in almond (Kester, unpublished).

The almond breeding program at Zaragoza has been directed to the obtention of self-compatible forms, basically with crosses involving the cultivar 'Tuono'. The results obtained so far and their analysis from the point of view of the transmission of self-compatibility are offered for discussion.

MATERIAL AND METHODS

The seedlings of the almond breeding program of Zaragoza are studied for self-compatibility as soon as the juvenile period is over and the first flowers are produced. Twenty flowers are collected and prepared for their pollination. If it is not possible to collect 20 flowers from one seedling at a time, this number can be accomplished through several collections. Several seedlings have only a few flowers the first year and the number of 20 flowers cannot be reached.

The flowers are emasculated and placed in a tray with water, with the peduncles through the holes of a plastic mesh floating over the water thanks to several pieces of wood or cork. This method saves space, and allows the study of individual flowers.

The pistils are pollinated when the pollen is dry (about two days) and the trays are placed in an oven at 22° C. The pistils are collected three days after-pollination, placed in a solution of 5 % Na_2SO_3, and autoclaved for 10 min at 1.2 kg cm^{-2}.

Table 1

*Number of seedlings in each class from the crosses
of the almond breeding program at Zaragoza*

Cross	Self-compatible	Self-incompatible	Intermediate
'Tuono'	48	2	8
'Tuono' × 'A-S-1'	5	—	2
'Tuono' × 'Genco'	1	3	—
Self-compatible × self-compatible	54	5	10
'Tuono' × 'Cristomorto'	4	—	1
'Tuono' × 'Fourcouronne'	1	8	4
'Tuono' × 'Ferragnès'	11	3	2
'Tuono' × 'Miagkoskorlupyj'	2	—	—
'Tuono' × 'Primorskij'	2	1	3
'Tuono' × 'Texas'	8	3	3
'Tuono' × 'Orés'	2	1	—
'Tuono' × 'Yaltinskij'	4	1	1
'Tuono' × 'T. de la Verdière'.........	5	—	—
'Tuono' × 'Marcona'	1	—	—
'Tuono' × ?	5	1	—
Self-compatible × self-incompatible	45	18	14
'T. de la Verdière' × 'Tuono'.........	7	—	2
'Titan' × 'Tuono'...................	10	3	7
Self-incompatible × self-compatible	17	3	9
'Tuono' × OP.......................	7	5	4
'A-S-1' × OP	5	8	2
'Genco' × OP......................	5	5	1
121 × OP	2	—	—
Self-compatible × OP...............	19	18	7

Pollen tube growth is assessed by observation in an UV microscope, by fluorescence of the callose deposits by aniline blue (Kho and Baer, 1968). Every seedling in studied at least during two consecutive years; if results are not consistent the study lasts longer. In this way the seedlings are classified as self-compatible or self-incompatible; however, some forms are classified as intermediate because some pistils (normally very few) have a single pollen tube at the base, and so are closer to the self-incompatible ones than to the self-compatible.

RESULTS AND DISCUSSION

Table 1 shows the results obtained up to 1982. It does not include the results from seedlings that have not been defined because of inconsistent results or of a short number of flowers produced so far. Table 2 transforms these results into percentages.

The small size of the offsprings in crosses with fruit trees leads to reduced numbers in the calculations, and so to their difficult mathematical interpretation. For this reason the typical X^2 analysis has not been applied. Besides, the presence of seedlings with a very long juvenile period means that these figures are incomplete. Nevertheless, a first approach can be tried.

In the gametophytic self-incompatibility system of almond, the changes are related to the S locus. Like any other gene, this locus can undergo mutations, naturally or artificially induced. A mutation can es-

Table 2

Percentage of seedlings in each class from the crosses
of the almond breeding program at Zaragoza

Cross	Self-compatible	Self-incompatible	Intermediate	Self-incompatible Intermediate
1: Self-compatible × self-compatible	78	7	14	22
2: Self-compatible × self-incompatible	58	23	18	41
3: Self-incompatible × self-compatible	58	10	31	41
4: Self-compatible × OP	43	40	16	56
2 + 3 + 4	54	26	20	46

tablish a self-campatible allele S_f (East, 1929), believed to be of the S series (Pandey, 1968), and the fixation of this allele in the offspring can establish a self-compatible system, though the mutation can revert to the self-incompatible situation (Lewis and Crowe, 1953).

Lewis and Crowe (1954) found different kinds of S mutations in cherry (a species very close to almond), with a permanent or reversible mutation affecting pollen activity and a permanent loss of the stylar activity; the two mutations can occur either independently or synchronously and can revert to normal independently. The induced mutations produce the same range of mutant types found in spontaneous mutations. These different kinds of S mutations induced these authors (1958), after studying the incompatibility behaviour in several plants, to suggest that three different steps occur in the mutation from self-incompatibility (SI) to self-compatibility (SC).

According to this theory (S_f allelic to the S series), as self-incompatible forms are found both among the offsprings of two self-compatible cultivars and among the progeny of one self-compatible and one self-incompatible cultivar, the present self-compatible cultivars used in these crosses must be heterozygous for self-compatibility.

However, as shown in Table 1, there is not a clear cut distinction between self-compatible and self-incompatible selections, because there are intermediate forms which are not clearly self-compatible but in some cases have pollen tubes growing through the whole style and reaching the ovules. This agrees with references to partial self-compatibility among almond cultivars (Almeida, 1945 and 1949; Bowman, 1939; Milella, 1959; Tufts, 1919), and almond selections derived from crosses with self-compatible culti-

vars (Grasselly, unpublished). These forms must be considered as agronomically self-incompatible as the few fruits obtained when self-pollinated are not a crop.

Environmental conditions, artificial treatments and genetic factors can affect self-incompatibility and be used to overcome it (Pandey, 1959), producing a small self set called pseudo-self-compatibility, first called pseudo-fertility by East and Park (1917). This could be the case in the intermediate forms mentioned above. But as environmental conditions are normal in the assays and no artificial treatments are applied, genetic factors can be supposed to affect self-compatibility in this way.

Grasselly (unpublished) has suggested the possibility that more than one locus could be implied in self-compatibility and its transmission. This could explain a deviation from 3:1 or 1:1 ratios among the offspring of different kinds of crosses, but could hardly explain the presence of intermediate forms. These may be due, as suggested by Pandey (1959), to a chance combination of modifier genes which weaken the incompatibility reaction. However, it is very difficult to consider modifier genes, as they are minor genes and are sometimes probably referred to to explain complex situations.

Kostina (1970), working with different ecological-geographical apricot populations possessing different degrees of self-compatibility, suggested that the inheritance of self-compatibility in apricot (otherwise a species very close to almond) may differ depending on the genetic characteristics of the initial forms. These comments might also apply to the almond selections, as observed in the results of different crosses, shown in Table 1: some crosses, as 'Tuono' x 'Tardive de la Verdiere' (in both ways), 'Tuono' x

'Cristomorto' (probably of a genetic background close to 'Tuono'), 'Tuono' x 'Ferragnes' (from a cross by 'Cristomorto'), have a higher proportion of self-compatible forms than the expected 1:1 ratio, but in other crosses, such as 'Tuono' x 'Fourcouronne', this proportion is lower. This has also been reported in crosses involving other cultivars by Grasselly (unpublished).

Socias i Company *et al.* (1976) found in almond selections derived from peach × almond hybridization different degrees of self-compatibility. They suggested that, even if the S_f allele from peach is allelic to the S series in almond, it could only be homoeologous and not completely homologous, with incomplete dominance in the hybrid offspring. Variations in the compatibility system could also occur because of the drastic change produced by shifting the S_f allele from a well coadapted system into a different well coadapted system which involves self-incompatibility. In addition, self-compatibility could be influenced by modifier genes influencing the major S locus and being manifested to different degrees in these hybrids when two different genetic systems are put together. Basides, they found female sterility due to ovule abortion in some of the selections.

'Tuono' has a very high proportion of flower sterility (Socias i Company, 1982), and various degrees of female sterility have been observed among its progenies (Socias i Company, unpublished). This parallels with the selections above mentioned studied by Socias i Company *et al.* (1976). The geographical distribution of the self-compatible cultivars is: Puglia (Italy), where most are found, as 'Tuono', 'Genco', 'Filippo Ceo' (Grasselly and Olivier, 1976; Herrero *et al.*, 1977), and also probably 'Duro Italiano' indicated by Almeida (1945) in Portugal and considered by

Grasselly (unpublished) very close to 'Tuono', and also 'Mazzetto' (Anonymous, 1972); Portugal, with 'José Dias' (Almeida, 1945); Spain, with 'A-S-1' (Herrero and Felipe, 1975); and Bulgaria, with 'Exinograd' (Costetchi, 1967). In Puglia and Bulgaria the species *P. webbii* Spach grows wild (Grasselly, 1976), and it probably grows also in Spain, in a region close to Portugal, and is self compatible (Felipe and Socias i Company, 1977). So, one can also consider the possibility of a natural transmission of self-compatibility from *P. webbii* to almond, producing a genetic unstability due to hybridization with female sterility and various degrees of self-compatibility.

It is possible to consider that almond is a self-incompatible species with a genetic background of pseudo-self-compatibility as indicated by the small self set observed in some cultivars. Above this background, only one S_f change breaks the incompatibility system. This S_f can be due to a natural mutation or to the transmission from a close species such as *P. webbii*. This S_f change must be recent because it has not been fixed, and probably the self-compatible forms are heterozygous for it because self-incompatible forms appear in their offspring. So, three classes are distinguished: self-compatible (new in the species), self-incompatible, and pseudo-self-compatible. The last two can be considered together, and examining the percentages of Table 2, where all the results have been pooled bearing this fact in mind, a relative closeness to the ratio 3 : 1 in the self-compatible × self-compatible crosses, and to 1 : 1 in the other crosses is observed. These results agree with the theory of gametophytic self-incompatibility in plants (Lewis and Crowe, 1958; Pandey, 1959 and 1968).

So one can conclude that self-compatibility in almond is probably due to a S_f allele in the S series, and that the forms studied so far are heterozygous.

BIBLIOGRAPHIE

ALMEIDA, C. R. Marqués de. 1945. Acerca da improdutividade na amendoeira. An. Inst. Agron. Lisboa. 15: 1-186.

ALMEIDA, C. R. Marqués de. 1949. Ainda acerca da improdutividade na amendoeira. An. Inst. Agron. Lisboa. 16: 51-71.

ANONYMOUS. 1972. Variétés fruitières et cépages à planter en Tunisie. Docum. Tech. Institut National de la Recherche Agronomique de Tunisie, 60, 69 pp.

BOWMAN, F. T. 1939. Pollination of fruit trees. J. Aust. Inst. Agric. Sci. 5 (4): 212-217.

COSTETCHI, M. 1967. Exinograd. In: Pomologia Republicii Socialiste Romania, Vol. VI, Ed. Academiei Republicii Socialiste Romania, Bucaresti, 490- 492.

CRANE, M. B.; BROWN, A. G. 1937. Incompatibility and sterility in the sweet cherry, Prunus avium L. J. Pomol. 15 (2): 86-116.

CROWE, L. K. 1964. The evolution of outbreeding in plants. I. The Angiosperms. Heredity 19 (3): 435-457.

EAST, E. M. 1929. Self-sterility. Bibliogr. Genet. 5: 331-368.

EAST, E. M.; MANGELSDORF, A. 1925. A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. USA 11 (2): 166-171.

EAST, E. M.; PARK, J. B. 1917. Studies on self-sterility. I. The behavior of self-sterile plants. Genetics 2 (6): 505-609.

FELIPE, A. J.; SOCIAS I COMPANY, R. 1977. Un amandier sauvage, probablement A. webbii, non encore mentionné en Espagne. 3e Colloque du GREMPA, Bari, 3-7 Octobre 1971, 78-79.

GRASSELLY, C. 1976. Les espèces sauvages d'amandiers. Options Méditerr. 32: 28-43.

GRASSELLY, C.; OLIVIER, G. 1976. Mise en évidence de quelques types autocompatibles parmi les cultivars d'amandier (P. amygdalus Batsch) de la population des Pouilles. Ann. Amélior. Plant. 26 (1): 107-113.

HERRERO, M.; CAMBRA, M.; FELIPE, A. J. 1977. Interpolinización de variedades de almendro. An. Inst. Nac. Invest. Agrar., Ser. Prod. Veg. 7: 99-103.

HERRERO, M.; FELIPE, A. J. 1975. Pollinisation de l'amandier. Incompatibilité pollen-style. 2e Colloque du GREMPA, Montpellier-Nimes, Septembre 1975.

KHO, Y. O.; BAER, J. 1968. Observing pollen tubes by means of fluorescence. Euphytica 17 (2): 298-302.

KOSTINA, K. F. 1970. (Investigations on apricot self-pollination). Tr. Gos. Nikit. Bot. Sad. 45: 7-17 (In Russian).

LEWIS, D.; CROWE, L. K. 1953. Theory of revertible mutations. Nature 172 (4376): 501.

LEWIS, D.; CROWE, L. K. 1954. Structure of the incompatibility gene. IV. Types of mutation in Prunus avium. L. Heredity 8 (3): 357-363.

LEWIS, D.; CROWE, L. K. 1958. Unilateral interspecific incompatibility in flowering plants. Heredity 12 (2): 233-256.

MILELLA, A. 1959. Ricerche sulla biologia fiorale del mandorlo in Sardegna. Riv. Ortoflorofruttic. Ital. 43 (7/8): 322-332.

PANDEY, K. K. 1959. Mutations of the self-incompatibility gene (S) and pseudocompatibility in angiosperms. Lloydia 22 (3): 222-234.

PANDEY, K. K. 1968. Compatibility relationships in flowering plants: role of the S-gene complex. Amer. Natur. 102 (927): 475-489.

SOCIAS I COMPANY, R. 1978. La autocompatibilidad en la mejora del almendro. I Congreso Internacional de Almendra y Avellana, Reus, 25-28 Octubre 1976, 513-522.

SOCIAS I COMPANY, R. 1982. Flower sterility in almond. Acta Hort. 139 (in press).

SOCIAS I COMPANY, R.; FELIPE, A. 1977. Heritability of self-compatibility in almond. 3e Colloque du GREMPA, Bari, 3-7 Octobre 1977. 181-183.

SOCIAS I COMPANY, R.; KESTER, D. E.; BRADLEY, M. V. 1976. Effects of temperature and genotype on pollen tube growth of some self-incompatible and self-compatible almond cultivars. J. Amer. Soc. Hort. Sci. 101 (5): 490-493.

TUFTS, W. P. 1919. Almond pollination. Calif. Agric. Sta. Bull. 306, 32 pp.