Challenges for Self-Compatibility Identification in Almond

R. Socias i Company, O. Kodad, A. Fernández i Martí and J.M. Alonso
Unidad de Fruticultura, CITA de Aragón
Av. Montañana 930, 50059 Zaragoza, Spain
rsocias@aragon.es

Keywords: *Prunus amygdalus* Batsch, breeding, S_f allele, allele identification, gene sequencing

Abstract

Several approaches have been utilised to assess the level of self-compatibility in almond, such as fruit set after self-pollination and bagging, pollen tube growth, and the more recent S_f allele identification by molecular markers and gene sequencing. However, none of these methods has given fully reliable results as all of them show advantages and limitations. An active S_f allele, not conferring self-compatibility in spite of its fully identity with the inactive S_f allele conferring self-compatibility, has been recently identified, showing that the presence of the S_f allele is not the only requirement for self-compatibility expression in almond and that the coding region of the S_f allele may not be involved in that expression. Missequencing of alleles has also created confusion for allele identification. Thus, a better knowledge of the plant material as a whole, and not only of its genotype, is fundamental for the understanding of self-compatibility in almond and for the evaluation of the new selections in a breeding programme.

INTRODUCTION

Although self-compatibility was discovered in almond as early as 1945 (Almeida, 1945), no attention was paid to the issue until the 1970s, when its importance in almond growing and in breeding for new self-compatible cultivars was fully understood (Socias i Company, 1978). The first attempts for self-compatibility identification involved fruit set evaluation after artificial self-pollinations (Almeida, 1945) because the importance of almond self-compatibility is basically horticultural, the obtaining of commercial yields after an acceptable fruit set.

Several approaches, each one showing advantages and limitations have been used to assess the level of self-compatibility. Effective self-compatibility implies, first at all, pollen tube growth after self- pollination similar to that after cross-pollination with cross-compatible pollen. Secondly, this good pollen tube growth after self-pollination should result in similar fruit sets, which may not always be the case. And thirdly, these fruit sets must reach the level of a commercial crop. From a horticultural point of view there is a fourth requirement, that these fruit sets must be obtained by autogamy, the ability of a genetically self-compatible cultivar to pollinate itself in the absence of insects (Weinbaum, 1985). Additionally, a good cultivar must always be productive with a crop of good kernel quality.

More recently, once the genetic structure of the S_f allele was further understood, the detection of self-compatibility has also been undertaken by molecular markers. Gametophytic self-compatibility such as in almond is controlled by a single polymorphic locus containing at least two linked genes, one specifically expressed in the pistil and the other in the pollen (Kao and Tsukamoto, 2004). The pistil component of this gene codes

for an *S*-RNase responsible for the pollen tube growth inhibition in the styles (Bošković et al., 1997). The candidate gene for the *S* pollen component (SFB) has been recently identified by Ushijima et al. (2003), showing a tight linkage with the *S*-RNase gene (Ikeda et al., 2005).

However, this information is only genetic, not horticultural, and the final evaluation of self-compatibility of a cultivar or selection is its productivity under field conditions, i.e., with solid blocks of one clone isolated from any other almond clone and even in the absence of pollinating insects. Thus, our objective was to review the different physiological and genetic aspects of almond self-compatibility in order to better understand its evaluation and application in an breeding programme, especially after that some confusing results have been obtained with S_f identification by molecular markers and gene sequencing.

FRUIT SET

The first results on almond self-compatibility were obtained by Almeida (1945 and 1949) by artificial self-pollinations. However, fruit set evaluation in the field is subjected to many environmental hazards in spite of being the most natural approach to the real self-compatibility level of any genotype. As a consequence, they show a very high variability between years (Socias i Company et al., 2004). Several results have shown that fruit set levels after self-pollination are not only related to the genetic self-compatibility of the selection under trial, but also to other genetic conditions of each genotype. This could be explained by the fact that almond is a self-incompatible species with a possible genetic background of pseudo-self-compatibility (Socias i Company, 1990), thus resulting in fruit set differences depending of the genotype, as well as to inbreeding depression (Alonso and Socias i Company, 2005). The differences between the results of different years point to unspecified environmental conditions affecting fruit set, stressing the need for self-compatibility evaluation in more than one year (Socias i Company et al., 2004).

In addition to the environmental conditions affecting natural fruit set in the field, as emasculation and pollination must be done in the open air, weather conditions are not always favourable to work because temperatures are usually very low at almond blooming time and if winds are blowing much attention must be paid to conduct the operations. Thus, fruit set determination in the field is mostly restricted to the final steps of self-compatibility evaluation in elite selections.

Although the level of fruit set has been stressed during the evaluation process in almond breeding (Oukabli et al., 2000; Socias i Company and Felipe, 1987 and 2007; Torre Grossa et al., 1994), it must be obtained by autogamy. Previous studies included bagging of branches (Grasselly and Olivier, 1984), or even enclosing whole trees in cages, with or without honey bees (Godini et al., 1994; Socias i Company and Felipe, 1992), but autogamy has only received attention recently (Dicenta et al., 2001; Godini et al., 1992; Kodad and Socias i Company, 2006; Socias i Company and Felipe, 1992; Socias i Company et al., 2004 and 2005; Vargas et al. 1998). This aspect is particularly important because only natural autogamy can allow solid plantings of one single cultivar, isolated from any other almond orchard and in the absence of pollinating insects. Flower morphology, in particular the relative positions of the stigma and anthers, is of great importance for natural autogamy (Bernad and Socias i Company, 1995; Godini et al., 1994; Kodad and Socias i Company, 2008; Socias i Company et al., 2004).

POLLEN TUBE GROWTH

Pollen tube growth is a clear indication of the compatibility of the pollination and as a consequence it has been repeatedly used in compatibility determinations since the first evaluation of self-compatibility in almond genotypes (Socias i Company et al., 1976). The flowers examined for assessing pollen tube growth can be kept in different environments as well as on the original branches or separated from them, giving the same unequivocal results (Socias i Company, 2001).

The studies conducted in the field show the most reliable response since they reflect the natural conditions of the pollination. However, these studies are subject to unpredictable weather conditions such as frosts. Frosts may destroy the pistils, but not so easily the pollen tubes, which growth is arrested by frosts, as well as by any low temperature (Socias i Company, 1982).

The problems to work in the field for pollen tube growth studies are the same than for fruit set evaluation. The weather contingencies may be avoided by taking whole branches to the laboratory or greenhouse and conducting emasculation and pollination on them, or by only taking single flower buds at stage D (Felipe, 1979), with which a great space saving is accomplished. In addition, the trays with the pollinated flowers can be maintained in chambers allowing controlling the temperature. Higher temperatures than usual increase the speed of compatible pollen tube growth whereas stress the symptoms of pollen incompatibility (Socias i Company et al., 1976).

Pollen tube growth studies have been often associated with fruit setting following artificial pollinations (Ben Njima and Socias i Company, 1995; Socias i Company and Felipe, 1987), giving concordant results. As well as for fruit set, inbreeding depression may affect the expression of self-compatibility by pollen tube growth (Alonso and Socias i Company, 2005).

RNASE ACTIVITY

Since Bošković and Tobutt (1996) reported that the *S* alleles code for stylar ribonucleases in cherry (*P. avium* L.) and that these RNases can be detected by separation of stylar proteins by non-equilibrium pH gradient electrofocusing (NePHGE) and subsequent staining for activity, the same approach was applied to almond *S* alleles. Bošković et al. (1999) found no RNase activity for the S_f allele, thus concluding that genotypes showing only one band for RNase activity were self-compatible. The absence of RNase activity may be due to the lack or to the very low level of the transcription of the *S*-RNase in the pistil (Hanada et al., 2009), as it was also reported in Japanese plum (*Prunus salicina* Lindl.) by Watari et al. (2007). However, the presence of one band is not enough to assess self-compatibility because inbreeding may produce an incompatible expression of self-compatible genotypes (Alonso and Socias i Company. 2005).

Some problems, however, have arisen when RNase detection has been applied to different genotypes, because two different RNase bands may coincide after electrophoresis separation, thus giving a wrong "one band" result when a real superposition of two bands is occurring. Consequently, this technique is only fully reliable for seedling identification when the genotypes of the two parents are previously known (Bošković et al., 2003).

ALLELE IDENTIFICATION AND SEQUENCING

The more recent advances in genetic analysis at the gene level have allowed a closer approach to the S_f allele in almond, both of the stylar and the pollen components.

First, *S* alleles, including S_f, were identified by PCR analysis using conserved and allele-specific primers (Channuntapipat et al., 2001; Ma and Oliveira, 2001). Later, the partial sequence of the S_f allele gene associated with S_f-RNase was obtained (Channuntapipat et al., 2001; Ma and Oliveira, 2001). Finally, Ushijima et al. (2003) sequenced the pollen *S* haplotype termed F-Box (SFB) finding that this could be a good candidate for the pollen *S* product since it was confirmed to be specifically expressed in the pollen tube and to be physically linked to the *S*-RNase gene (Entani et al., 2003, Ikeda et al., 2005). This identification was in self-incompatible almond genotypes, but later the self-compatible SFB_f was sequenced by Bošković et al. (2007) and Hanada et al. (2009).

Various consensus primer sets have been designed from conserved regions of *S*-genes to amplify across the second intron (Channuntapipat et al., 2003; Tamura et al., 2000), the first intron (Ortega et al., 2005), or both (Sutherland et al., 2004), to determinate *S*-genotypes in almond. However, PCR primers designed from conserved regions do not always distinguish between alleles with a similar number of nucleotides (López et al., 2004). In addition, other primer sets were designed specifically to amplify some *S* genes, including S_f (Channuntapipat et al., 2001; Ma and Oliveira, 2001). PCR-based markers of almond *S*-alleles have been employed to facilitate the integration of self-compatible *S*-alleles from related species (Gradziel et al., 2001). Screening efficiency and flexibility have been greatly increased with the development of successful multiplex PCR techniques by Sánchez Pérez et al. (2004).

Since the beginning, several amino acid sequences for the S_f-RNase have been deposited in the database by different authors. When all these sequences are compared, several differences are observed between them, thus raising the question of mistakes in sequencing because most of allele sequences have been determined in 'Tuono' and genotypes derived from it, consequently for the same S_f-RNase and discarding different sources of self-compatibility for the genotypes studied. Already the first sequences by Channuntapipat et al. (2001) and Ma and Oliveira (2001) were different, although further results suggest that the sequence by Channuntapipat et al. (2001) is correct and must be taken as the consensus one.

Fig. 1 shows the alignment of the published sequences for the S_f-RNase as well as some other *S*-RNases for comparison. The first one (AY291117) was amplified in 'Lauranne' and selection IRTA12-2, two self-compatible genotypes deriving from 'Tuono' (Channuntapipat et al., 2001). In spite of the origin of these genotypes, it is only 98% identical to the 'Tuono' sequence (AF157009) by Ma and Oliveira (2001), 64% to the 'Tuono' sequence (DQ156217) by Barckley et al. (2006), and 99.3% to the 'Tuono' (AM690356) sequence by Bošković et al. (2007). It is identical to the S_f sequence of 'Cambra' (EU684318), a cultivar derived from 'Tuono' (Kodad et al., 2009), but also to two *S* alleles reportedly conferring self-incompatibility in almond, S_{fa} of 'Ponç' (EU293146; Kodad et al., 2009) and S_{30} of 'Fra Giulio Grande' (AM690361; Bošković et al., 2007).

These results show that some missequencings and misinterpretations occurred during allele analysis. Ma and Oliveira (2001) showed valine instead of isoleucine and histidine instead of arginine in the C2 region, probably as a result of a mistake in sequencing. Bošković et al. (2007) had to recognize a missequencing in a note added in proof, thus invalidating most of the reasoning of their conclusions, as their 'Tuono' S_f did not really show the supposed histidine substitution instead of arginine in its sequence. Barckley et al. (2006) gave an amino acid sequence for 'Tuono' S_f identical to S_1, probably due to missampling and showing that the 'Tuono' genotype present in California

must be the same than that present in other countries and utilized for the other analysis, as opposed to their original suggestion.

These mistakes led to Bošković et al. (2007) to incorrectly name a new allele, S_{30}, which is identical to S_f, although showing a different activity (Kodad et al., 2009). This new name may create new confusions in almond *S* allele research because the identity of the S_f allele must be preserved, in spite of showing two different phenotypical expressions. As a consequence, Kodad et al (2009) have suggested the denomination S_{fa} for the active S_f allele showing a self-incompatible expression.

The identification of the SFB_f allele has not shown these problems, as the sequences for 'Tuono' (AM711126) by Bošković et al. (2007) and for 'Lauranne' (AB361036) by Hanada et al. (2009) are identical. This sequence is also the same than for the self-incompatible SFB_f allele sequenced in 'Ponç' (EU310402) by Kodad et al. (2009) and in 'Fra Giulio Grande' (AM711127) by Bošković et al. (2007). These coincidences indicate that the SFB_f allele, as well as the S_f-RNase allele may have two different phenotypical expressions, and that both the pistil and the pollen components of the S_f allele show the same compatible or incompatible behaviour simultaneously. Thus the coding region of the S_f gene may not be the exclusive origin of self-compatibility in almond (Kodad et al., 2009), and that some genetic modification outside this coding region is affecting that expression (Fernández i Martí et al., 2009).

CONCLUSIONS

The different approaches applied to identify self-compatibility in almond show advantages and limitations. Especially missequencing of alleles has created confusion for allele identification. The recent identification of an active S_f allele, not conferring self-compatibility in spite of its fully identity with the inactive S_f allele, that so far considered as the allele conferring self-compatibility, shows that the presence of the S_f allele is not the only requirement for self-compatibility expression in almond and that the coding region of the S_f allele may not be involved in that expression. The knowledge of only the genotype is not enough in almond self-compatibility research, making fundamental a global study of the plant material for the evaluation of the real ability of any genotype to set fruit under autogamy conditions, thus allowing its further selection as a registered cultivar.

ACKNOWLEDGEMENTS

This research was supported by the grant AGL2007-65853-C02-02 of the Spanish CICYT and the Consolidated Research Group A12 of Aragón.

Literature Cited

Almeida, C.R. Marques de. 1945. Âcerca da improdutividade na amendoeira. An Inst. Agron. Lisboa 15:1-186.

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

Alonso, J.M. and Socias i Company, R. 2005. Self-compatibility expression in self-compatible almond genotypes may be due to inbreeding. J. Amer. Soc. Hort. Sci. 130:868-869.

Barckley, K.K., Uratsu, S.L., Gradziel T.M. and Dandekar, A.M. 2006. Multidimensional analysis of *S*-alleles from cross-incompatible groups of California almond cultivars. J. Amer. Soc. Hort. Sci. 131:632-636.

Ben Njima, N. and Socias i Company, R. 1995. Characterization of some self-compatible almonds. I. Pollen tube growth. HortScience 30:318-320.

Bernad, D. and Socias i Company, R. 1995. Characterization of some self-compatible almonds. II. Flower phenology and morphology. HortScience 30:321-324.

Bošković, R. and Tobutt, K.R. 1996. Correlation of stylar ribonuclease zymograms with incompatibility alleles in sweet cherry. Euphytica 90:245-250.

Bošković, R., Tobutt, K.R., Batlle, I. and Duval, H. 1997: Correlation of ribonuclease zymograms and incompatibility genotypes in almond. Euphytica 97:167-176.

Bošković, R., Tobutt, K.R., Duval, H., Batlle, I., Dicenta, F. and Vargas, F.J. 1999. A stylar ribonuclease assay to detect self-compatible seedlings in almond progenies. Theor. Appl. Genet. 99:800-810.

Bošković, R., Tobutt, K.R., Batlle, I., Duval, H., Martínez-Gómez, P.and Gradziel, T.M. 2003. Stylar ribonuclease in almond: correlation with and prediction of incompatibility genotypes. Plant Breed. 122:70-76.

Bošković, R., Tobutt, K.R., Ortega, E., Sutherland, B.C. and Godini, A. 2007. Self-(in)compatibility of the almond *P. dulcis* and *P. webbii*: detection and cloning of 'wild-type S_f' and new self-compatibility alleles encoding inactive *S*-RNases. Mol. Genet. Genomics 278:665-676.

Channuntapipat, C., Sedgley, M. and Collins, G. 2001. Sequences of the cDNAs and genomic DNAs encoding the *S1, S7, S8* and *Sf* alleles from almond, *Prunus dulcis*. Theor. Appl. Genet. 103:1115-1122.

Channuntapipat, C., Wirthensohn, M., Ramesh, S.A., Batlle, I., Arús, P., Sedgley, M. and Collins, G. 2003: Identification of incompatibility genotypes in almond (*Prunus dulcis* Mill.) using specific primers based on the introns of the S-alleles. Plant Breed. 122:164-168.

Dicenta, F., Ortega, E., Cánovas, J.A. and Egea, J. 2001. Self-pollination versus cross-pollination of six self-compatible almond cultivars: pollen tube growth and fruit set. Cah. Options Méditerr. 56:369-372.

Entani, T., Iwano, M., Shiba, H., Che, F.S., Isogai, A. and Takayama, S. 2003. Comparative analysis of the self-incompatibility (*S*-) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic diversity. Genes Cells 8:203-213.

Felipe, A.J. 1977. Almendro. Estados fenológicos. Inf. Técn. Econ. Agrar. 27:8-9.

Fernández i Martí, A., Hanada, T., Alonso, J.M., Yamane, H., Tao, R. and Socias i Company, R. 2009. A modifier locus affecting the expression of the *S*–RNase gene could be the cause of breakdown of self-incompatibility in almond. Sex. Plant Reprod. 22:179-186.

Godini, A., de Palma, L. and Palasciano, M. 1992. Role of self-pollination and reciprocal stigma/anthers position on fruit set of eight self-compatible almonds. HortScience 27:887-889.

Godini, A., de Palma, L. and Palasciano, M. 1994. Self-fertile almonds and fruit set by optimized self- and cross-pollination. Acta Hort. 373:157-160.

Gradziel, T.M., Martínez-Gómez, P., Dicenta, F. and Kester, D.E. 2001. The utilization of related almond species for almond variety improvement. J. Amer. Pomol. Soc. 55:100-108.

Grasselly, C. and Olivier G., 1984. Avancement du programme de création de variétés autocompatibles. Options Méditerr. CIHEAM/IAMZ 84/II:129-131.

Hanada, T., Fukuta, K., Yamane, H., Esumi, T., Gradziel, T.M., Dandekar, A.M., Fernández i Martí, A., Alonso, J.M., Socias i Company, R. and Tao, R. 2009: Cloning and characterization of self-compatible S_f haplotype in almond (*Prunus dulcis* (Mill.) D.A. Webb.). HortScience 44:609-613.

Ikeda, K., Ushijima, K., Yamane, H., Tao, R., Hauck, N.R., Sebolt, A.M. and Iezzoni, A.F. 2005. Linkage and physical distances between the *S*-haplotype *S*-RNase and *SFB* genes in sweet cherry. Sex. Plant Reprod. 17:289–296.

Kao, T-H. and Tsukamato, T. 2004. The molecular and genetic bases of *S*-RNase-based self-incompatibility. Plant Cell 16:72-83.

Kodad, O. and Socias i Company, R. 2006. Pollen source effect on pollen tube growth in advanced self-compatible almond selections (*Prunus amygdalus* Batsch). Adv. Hort. Sci. 20: 256-261.

Kodad, O. and Socias i Company, R. 2008. Fruit set evaluation for self-compatibility selection in almond. Scientia Hort. 118:260-265.

Kodad, O., Socias i Company, R., Sánchez, A. and Oliveira, M. 2009: The expression of self-compatibility in almond may not only be due to the presence of the S_f allele. J. Amer. Soc. Hort. Sci. 134: 221-227.

López, M., Mnejja, M., Rovira, M., Collins, G., Vargas F.J., Arús, P. and Batlle, I. 2004. Self-incompatibility genotypes in almond re-evaluated by PCR, stylar ribonucleases, sequencing analysis and controlled pollinations. Theor. Appl. Genet. 109:954-964.

Ma, R.C. and Oliveira, M.M., 2001. The RNase PD2 gene of almond (*Prunus dulcis*) represents an evolutionary distinct class of S-like RNase genes. Mol. Gen. Genet. 263:925-933.

Ortega, E., Sutherland, B.G., Dicenta, F., Bošković, R. and Tobutt, K.R. 2005. Determination of incompatibility genotypes in almond using first and second intron consensus primers: detection of new *S* alleles and correction of reported *S* genotypes. Plant Breed. 124:188–196.

Oukabli, A., Lansari, A., Wallali, D.L., Abousalim, A., Egea, J. and Michaux-Ferriere, N. 2000. Self and cross pollination effects on pollen tube growth and fertilization in self-compatible almond *Prunus dulcis* 'Tuono'. J. Hort. Sci. Biotechnol. 75:739-744.

Sánchez-Pérez, R., Dicenta, F. and Martínez-Gómez, P. 2004. Identification of *S*-alleles in almond using multiplex-PCR. Euphytica 138:263-269.

Socias i Company, R. 1978. La autocompatibilidad en la mejora del almendro. I Congreso Internacional de Almendra y Avellana, Reus, 25-28 octubre 1976, pp. 513-522.

Socias i Company, R. 1982. Ensayo de polinización de diferentes clones del almendro 'Marcona'. An. Inst. Nac. Invest. Agrar., Ser. Agric. 19: 11-21.

Socias i Company, R. 2001. Differential growth of almond pollen tubes in three environments. Cah. Options Méditerr. 56: 59-64.

Socias i Company, R. and Felipe, A.J. 1987. Pollen tube growth and fruit set in a self-compatible almond selection. HortScience 22:113-116.

Socias i Company, R. and Felipe, A.J. 1992. Self-compatibility and autogamy in 'Guara' almond. J. Hort. Sci. 67:313-317.

Socias i Company, R. and Felipe, A.J. 2007. 'Belona' and 'Soleta' almonds. HortScience 42 (3): 704-706.

Socias i Company, R., Kester, D.E. and Bradley, M.V. 1976. Effects of temperature and genotype on pollen tube growth of some self-compatible and self-incompatible almond cultivars. J. Amer. Soc. Hort. Sci. 101:490-493.

Socias i Company, R., Alonso, J.M. and Gómez Aparisi, J. 2004. Fruit set and productivity in almond as related to self-compatibility, flower morphology and bud density. J. Hort. Sci. Biotechnol. 79:754-758.

Socias i Company, R., Gómez Aparisi, J. and Alonso, J.M. 2005. Year and enclosure effects on fruit set in an autogamous almond. Scientia Hort. 104: 369-377.

Sutherland, B.G., Robbins, T.P. and Tobutt, K.R. 2004. Primers amplifying a range of *Prunus S*-alleles. Plant Breed. 123:582-584.

Tamura, M., Ushijima, K., Sassa, H., Hirano, H., Tao, R., Gradziel, T.M. and Dandekar, A.M. 2000. Identification of self-incompatibility genotype of almond by allele-specific PCR analysis. Theor. Appl. Genet. 101:344-349.

Torre Grossa, J.P., Vaissière, B.E., Rodet, G., Botella, L. and Cousin, M. 1994. Besoins en pollinisation de la variété d'amandier auto-compatible "Lauranne". Acta Hort. 373:145-152.

Ushijima, K., Sassa, H., Dandekar, A.M., Gradziel, T.M., Tao, R. and Hirano, H. 2003. Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed F-Box gene with haplotype-specific polymorphism. Plant Cell 15:771-781.

Vargas, F.J., Clavé, J., Romero, M., Batlle, I. and Rovira, M. 1998. Autogamy studies on almond progenies. Acta Hort. 470:74-81.

Watari, A., Hanada, T., Yamane, H., Esumi, T., Tao, R., Yaegaki, H., Yamaguchi, M., Beppu, B. and Kataoka, I. 2007. A low transcriptional level of S^e-*RNase* in the S^e-haplotype confers self-compatibility in Japanese plum**.** J. Amer. Soc. Hort. Sci. 132:396-406.

Weinbaum, S.A. 1985. Role of natural self-pollination in self-fruitfulness in almond. Scientia Hort. 27:295-302.

Figures

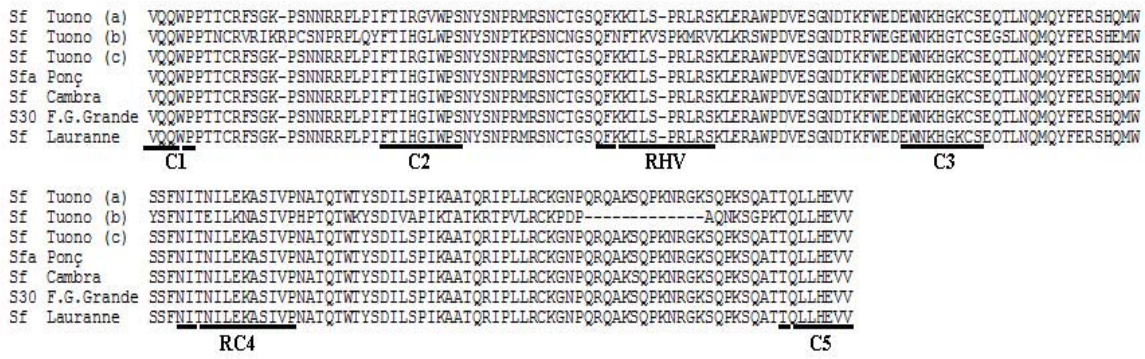

Fig. 1: Multiple alignment of the deduced amino acid sequence of different *S* almond alleles. Accession numbers are as follows: S_f of 'Tuono' a (AF157009; Ma and Oliveira, 2001); S_f of 'Tuono' b (DQ156217; Barckley et al., 2006); S_f of 'Tuono' c (AM690356, Bošković et al., 2007); S_{fa} of 'Ponç' (EU293146, Kodad et al., 2009); S_f of 'Cambra' (EU684318, Kodad et al., 2009); S_{30} of 'Fra Giulio Grande' (AM690361, Bošković et al., 2007); S_f of 'Lauranne' (AY291117; Channuntapipat et al., 2001).