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Significance of *S*-genotype determination in the conservation of genetic resources and breeding of almond

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Abstract. Most almonds are self-incompatible, and they are also cross-incompatible with those having an identical incompatibility (*S*) genotype. In recent years, several almonds have been *S*-genotyped using different approaches. This information has been included in different tables of cross-incompatibility groups, which are an update of the previously proposed one. However, no attempt has been made to reconcile the information of all these tables neither to correct possible inconsistencies. In this work we have determined the *S*-genotype of 15 Spanish almond cultivars previously un-genotyped using consensus primers for the first and second introns of *Prunus S*-RNases, and also allele-specific primers here designed. We could identify a new *S*-RNase allele numbered as S_{51}, which was cloned and sequenced. With the information obtained in this work and in previous studies, we have established eight new cross-incompatibility groups. Some of the already proposed groups need to be re-numbered to avoid the gaps left by those no longer existing. The determination of the *S*-genotype of almond genetic resources to be preserved as part of the agricultural biodiversity will facilitate the use of this material in research and breeding, and also provides useful information about phylogenetic relationships.

Keywords. *Prunus dulcis* – Genetic resources – Cross-incompatibility – *S*-genotyping.

Importance de la détermination du génotype S dans la conservation des ressources génétiques et la sélection d'amande

Résumé. *La plupart des amandes sont auto-incompatibles, ils sont aussi cross-incompatibles avec celles ayant un génotype (S) d'incompatibilité identiques. Au cours des dernières années, plusieurs amandes ont été S-génotypés en utilisant différentes approches. Cette information a été incluse dans différents tables des groupes cross-incompatibilité, qui sont une mise à jour de ceux proposés précédemment. Cependant, aucune tentative n'a été faite pour concilier les informations de toutes ces tables ni de corriger les éventuelles incohérences. Dans ce travail, nous avons déterminé le S-génotype de 15 cultivars d'amandier espagnols précédemment non-génotypés en utilisant des amorces consensus pour les premier et seconde introns de S-RNases de* Prunus*, mais aussi des amorces allèle-spécifiques ont été conçus. Nous avons pu identifier un nouvel allèle S-RNase numéroté comme S_{51}, qui a été cloné et séquencé. Avec les informations obtenues dans ce travail et dans les études précédentes, nous avons établi huit nouveaux groupes cross-incompatibilité. Certains des groupes déjà proposés doivent être re-numéroté pour éviter les lacunes laissés par ceux qui n'existe plus. Le S-génotypage des ressources génétiques d'amande préservée dans le cadre de la biodiversité agricole facilitera l'utilisation de ce matériau dans la recherche et la sélection, et fournira également des informations utiles sur les relations phylogénétiques.*

Mots-clés. Prunus dulcis – *Ressources génétiques – Cross-incompatibilité – S-génotypage.*

I – Introduction

Most almond cultivars [*Prunus dulcis* (Mill.) D.A. Webb] are self-incompatible, and they are cross-incompatible with those cultivars with the same incompatibility genotype. Self-incompatibility in almond is of the gametophytic type and it is controlled by the *S* locus, which has two genes with expression in pistil (S-RNases) and in pollen (SFB) (Tao and Iezzoni, 2010). The *S*-genotype of several almond cultivars has been determined initially by controlled crosses and later by isoelectric focusing and PCR (Kester *et al.*, 1994; Bošković *et al.*, 2003; Ortega *et al.*, 2005; Halász *et al.*, 2010; Kodad *et al*., 2010). The information obtained with the different methods indicated has been included in different versions of a table of cross-incompatibility groups (CIGs) (Bošković *et al.*, 2003; López *et al*., 2006; Ortega *et al*., 2006; Valizadeh *et al.*, 2009; Halász *et al.,* 2010; Kodad *et al*., 2010; Mousavi *et al*., 2011; Hafizi *et al*., 2013). Each of these tables is essentially an update of the previously proposed one. However, no attempt has been made to reconcile the information of all these tables neither to correct possible inconsistencies. Knowledge of the CIGs is very useful in breeding programs and it makes easier the design of plantations.

As part of a complete agronomical characterization of almond genetic resources grown in an open field genebank, we aimed to determine the *S*-genotype of 15 cultivars. We also aimed to establish cross-incompatibility groups with the information of this and previous studies.

II – Materials and methods

Young leaves were collected from trees of 15 Spanish almond cultivars previously un-genotyped for self-incompatibility and cultivated at the collection of IRTA (Tarragona, Spain). Leaf samples were also collected from 15 almond cultivars and one selection grown at the experimental field of CEBAS-CSIC (Murcia, Spain), which were used as reference for the almond *S*-alleles S_1-S_{29} and S_f described in Ortega *et al.* (2005).

Genomic DNA was extracted from leaf samples following the CTAB protocol described in Sonneveld *et al*. (2001). *S*-genotypes were identified by PCR of *S*-RNases using the consensus primers EM-PC2consFD + EM-PC3consRD and PaConsI-F + EM-PC1consRD as indicated in Ortega *et al.* (2005). Moreover, in a few particular cases, PCR with allele specific primers for S_3 or S_{10} designed in the present study was performed. When the PCR products generated using the sets of primers above indicated differed in size from those of the reference *S*-RNase alleles they were considered to correspond to potentially new *S*-RNase alleles, and they were therefore cloned and sequenced to ensure their identity. For this, the region between the signal peptide and the conserved region C5 was amplified from genomic DNA using the PCR reaction and cycling parameters indicated in Ortega *et al.* (2006). Purification of PCR products, cloning, transformation and selection of positive clones were as detailed in Ortega *et al.* (2006). For each *S*-RNase allele, three plasmids were sent for sequencing to STAB VIDA (Caparica, Portugal) using the M13 primers. The identity of the sequences obtained was ascertained by comparison with those available in the European Bioinformatics Institute web site (http://www.ebi.ac.uk).

III – Results and discussion

1. *S*-genotyping and new *S*-RNase

Using the different sets of primers above indicated, most of the 15 almond cultivars could be *S*-genotyped (Table 1). However, in two of the cultivars cloning and sequencing of the *S*-RNase alleles were necessary to complete the characterisation. In this manner, the presence of S_{35}, and of S_{24} and a new *S*-RNase allele named S_{51} was determined in 'Parque Samá' and 'Mollar de la Princesa', respectively. As observed in Table 1, the most frequent alleles were S_{10}, S_{12} and S_{27}.

2. Establishment of cross-incompatibility groups

Eight new CIGs were established after drawing together the results of this work and the information included in the previously proposed tables of CIGs (Bošković *et al.*, 2003; López *et al*., 2006; Ortega *et al*., 2006; Valizadeh *et al.*, 2009; Halász *et al.,* 2010; Kodad *et al*., 2010; Mousavi *et al*., 2011; Hafizi *et al*., 2013). The new CIGs XLI and XLII are formed by cultivars with S_4S_{13} and S_3S_9 genotype, respectively. The other six CIGs, to which the cultivars *S*-genotyped in this work belong to, are indicated in Table 1. In addition, some of the already proposed groups were re-numbered to avoid the gaps left by those no longer existing. It is noteworthy that, in the literature, accessions with the same name had a different *S*-genotype, what needs to be checked to ascertain which one is the correct.

Table 1. PCR product sizes with second and first intron consensus primers, allele-specific PCR scores, *S*-genotype and cross-incompatibility group (CIG) assessed to 15 Spanish almond cultivars

Cultivar	Second intron product size (bp)	First intron product size (bp)[a]	Allele-specific PCR		*S*-genotype	CIG
			S_3	S_{10}		
'Angones'	300, 1130	n.a., 400		+	$S_{10}S_{22}$	O
'Asperilla'	300, 1360	n.a., 380		+	$S_{10}S_{27}$	O
'Belardino'	450, 400				S_2S_{11}	O
'Caima'	350, 1300	n.a., 200		–	S_5S_{12}	XLVIII**
'Carreró'	1300, 1360	200, 380			$S_{12}S_{27}$	XLVII**
'Esperanza Forta'	1300, 1130	200, 400			$S_{12}S_{22}$	XLIII**
'Mollar de la Princesa'*	875, 570		–		$S_{24}S_{51}$	O
'Mollar de Tarragona'	750, 1300	590/700/870, 200			S_1S_{12}	XLVI**
'Nano'	1300, 340	200, 300			$S_{12}S_{28}$	XLIV**
'Parque Samá'*	750, 1280	590/700/870, 380			S_1S_{35}	O
'Pauet'	570, 300	350, n.a.		+	S_6S_{10}	XLV**
'Pep de Juneda'	750, 300	590/700/870, n.a.		+	S_1S_{10}	XIII
'Rof'	330, 690	n.a., 375	–		S_5S_{23}	O
'Tardaneta'	330, 1300	n.a., 200	–		S_5S_{12}	XLVIII**
'Verd'	1300, 1360	200, 380			$S_{12}S_{27}$	XLVII**

[a] n.a.: no amplification.

* *S*-genotype determined after sequencing.

** CIG established in this work.

The determination of the *S*-genotype of almond genetic resources to be preserved as part of the agricultural biodiversity will facilitate the use of this material in research and breeding. Moreover, S-genotyping allows detecting the frequency of the *S* alleles in local populations, what provides useful information about phylogenetic relationships.

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