Methylation of the S_f locus in almond is associated with *S*-RNase loss of function

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Self-compatibility (SC) in almond (*Prunus amygdalus* Batsch) is attributed to the presence of the S_f haplotype. Some forms of the S_f haplotype, however, are phenotypically self-incompatible (SI) even though their nucleotide sequences are identical. DNA from leaves and styles from genetically diverse almond samples was cloned and sequenced and then analyzed for changes affecting S_f variants. The cultivars used were Blanquerna (SC), Vivot (SI), Ponç (SI), Soleta (SC), M-2-16 (SC) and A-2-199 (SC). Once DNA was extracted, it was submitted to the DNA bisulphite modification treatment 'MethylEasy'. Epigenetic changes in several cytosine residues were detected in a fragment of 4700 bp of the *5'* upstream region of all SC samples of the S_f allele, differentiating them from all SI samples of S_f analyzed. When the S_f-RNase sequence is methylated, its expression is inhibited resulting in an SC phenotype, as occurs in 'Blanquerna', 'Soleta' and the two homozygous SC selections (M-2-16 & A-2-199). However, when S_f-RNase sequences do not show methylated cytosines, the RNase remains active, resulting in a SI genotype and phenotype, as in 'Vivot' and 'Ponç'.

This is the first report of DNA methylation in a Rosaceae species and appears to be strongly associated with inactivation of the S_f allele (Fernandez i Marti et al., 2014). Results facilitate an understanding of the evolution of SC/SI in almond and other *Prunus* species, and suggest novel approaches for future crop improvement.

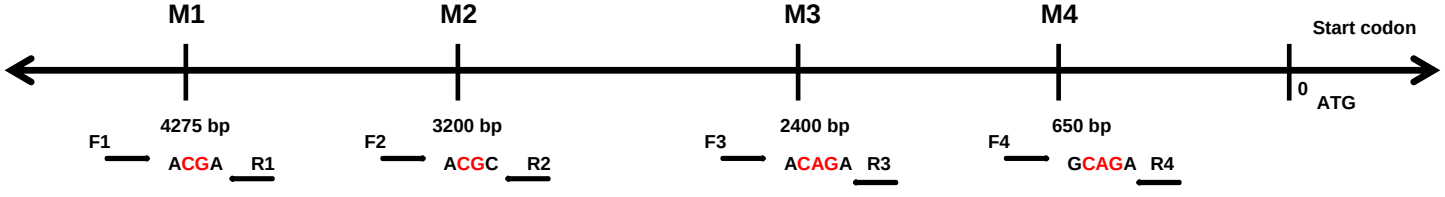

Fig. 1: Diagram showing the detection of methylated sites in the 5′ untranslational region of *Sf*-RNase. Primer positions overlapping the cytosine residues are indicated by *black arrows*. Methylated cytosines, either detected in the CG or the CNG forms, are indicated in *red*

A

	Blanquerna (SC) M1	Soleta (SC) M1	A2-199 (SC) M1	M2-16 (SC) M1	Vivot (SI) M1	Ponç (SI) M1
Clone 1	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTATGAAATTGGGG
Clone 2	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTATGAAATTGGGG
Clone 3	TGGTTTACGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTATGAAATTGGGG
Clone 4	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTATGAAATTGGGG
Clone 5	TGGTTTACGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTACGAAATTGGGG	TGGTTTATGAAATTGGGG	TGGTTTATGAAATTGGGG

B

	Blanquerna (SC) M2	Soleta (SC) M2	A2-199 (SC) M2	M2-16 (SC) M2	Vivot (SI) M2	Ponç (SI) M2
Clone 1	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGATGTTTAAATT	GATAAGATGTTTAAATT
Clone 2	GATAAGACGTTTAAATT	GATAAGATGTTTAAATT	GATAAGACGTTTAAATT	GATAAGATGTTTAAATT	GATAAGATGTTTAAATT	GATAAGATGTTTAAATT
Clone 3	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGATGTTTAAATT	GATAAGATGTTTAAATT
Clone 4	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGATGTTTAAATT	GATAAGATGTTTAAATT
Clone 5	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGACGTTTAAATT	GATAAGATGTTTAAATT	GATAAGATGTTTAAATT

C

	Blanquerna (SC) M3	Soleta (SC) M3	A2-199 (SC) M3	M2-16 (SC) M3	Vivot (SI) M3	Ponç (SI) M3
Clone 1	TTGATAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGATAGAGATTGTTGT
Clone 2	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGATAGAGATTGTTGT
Clone 3	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGATAGAGATTGTTGT
Clone 4	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGATAGAGATTGTTGT
Clone 5	TTGACAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGACAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGATAGAGATTGTTGT	TTGATAGAGATTGTTGT

D

	Blanquerna (SC) M4	Soleta (SC) M4	A2-199 (SC) M4	M2-16 (SC) M4	Vivot (SI) M4	Ponç (SI) M4
Clone 1	ATTAAAGTAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGTAGAGAGTATA
Clone 2	ATTAAAGCAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGTAGAGAGTATA
Clone 3	ATTAAAGCAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGTAGAGAGTATA
Clone 4	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGTAGAGAGTATA
Clone 5	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGCAGAGAGTATA	ATTAAAGTAGAGAGTATA	ATTAAAGTAGAGAGTATA

Fig. 2: Sequences surrounding the four (**a–d**) methylated points in the six genotypes studied. For each genotype and region five clones were sequenced. All samples were from leaves collected in Saragossa in the spring

A Fernández i Martí, T Gradziel and R Socias i Company (2014). Methylation of the S_f locus in almond is associated with S-RNase loss of function. Plant Molecular Biology 86 (6): 681-689