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Basic RNases of wild almond (*Prunus webbii*): Cloning and characterization of six new *S*-RNase and one "non-*S* RNase" genes

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Summary

In order to investigate the *S-RNase* allele structure of a *Prunus webbii* population from the Montenegrin region of the Balkans, we analyzed 10 *Prunus webbii* accessions. We detected 10 different *S-RNase* allelic variants and obtained the nucleotide sequences for six *S-RNase*s. The BLAST analysis showed that these six sequences were new *Prunus webbii S-RNase* alleles. It also revealed that one of sequenced alleles, S_9-*RNase*, coded for an amino acid sequence identical to that for *Prunus dulcis* S_{14}-RNase, except for a single conservative amino acid replacement in the signal peptide region. Another, S_3-RNase, was shown to differ by only three amino acid residues from *Prunus salicina* Se-RNase. The allele S_7-*RNase* was found to be inactive by stylar protein isoelectric focusing followed by RNase-specific staining, but the reason for the inactivity was not at the coding sequence level. Further, in five of the 10 analyzed accessions, we detected the presence of one active basic RNase (marked PW_1) that did not amplify with *S*-RNase-specific DNA primers. However, it was amplified with primers designed from the PA1 RNase nucleotide sequence (basic "non-*S* RNase" of *Prunus avium*) and the obtained sequence showed high homology (80%) with the PA1 allele. Although homologs of PA1 "non-*S* RNases" have been reported in four other *Prunus* species, this is the first recorded homolog in *Prunus webbii*. The evolutionary implications of the data are discussed.

Abbreviations: GSI, gametophyte self-incompatibility; SC, self-compatibility; SI, self-incompatibility.

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Introduction

Angiosperms with hermaphrodite flowers avoid inbreeding through the activity of self-incompatibility (SI) systems. One of the two known SI systems is gametophyte self-incompatibility (GSI), which provides distinction of self from non-self-pollen by detection of the pollen *S*-genotype and leads to abortion of self-pollen tube growth. The *S*-RNase-based GSI system has been investigated in the families *Rosaceae* (to which the genus *Prunus* belongs), *Solanaceae* and *Scrophulariaceae* and shown to be under the control of a highly polymorphic genome region, termed the *S*-locus. The *S*-locus comprises two genes encoding of female and male constituents of self/non-self distinction. The female (stylar) component of self-pollen recognition is a small, basic glycoprotein (p*I* = 8–10) with ribonuclease activity (McClure et al., 1989; Lee et al., 1994), therefore named *S*-RNase. Recently, the male (pollen) component of the GSI system was identified as a small protein, with an F-box region in the N terminus and named *S*-locus F-box (SFB) protein (Ushijima et al., 2003). In addition to *S-RNase* and *SFB* genes, experimental findings have indicated that non-*S*-linked genes, or modifier genes, are also included in GSI. Modifier genes most likely create a ''background'' for the SI reaction, affecting *S*-allele expression directly or through interaction with *S*-locus products, influencing the final outcome of pollination (for review see Roalson and McCubbin, 2003).

S-RNases are small basic glycoproteins with an essentially conservative structure. Sharing two conserved sequence motifs (including histidine residues responsible for the RNase function) and similar topology (due to preserved cysteine residues) with RNase T2 found in fungi, they are classified in an RNase superfamily named T2/S-type RNases. In the amino acid sequence, the signal peptide region is followed by five evolutionarily conserved regions (C1–C5) homologous among three families (*Rosaceae*, *Solanaceae* and *Scrophulariaceae*), with the exception of region C4, which is specific to *Rosaceae* and therefore named RC4 (Ushijima et al., 1998). Although common to all three families, the structure of the *S-RNase* gene shows several differences. While the rosaceous *S-RNase* gene contains one hypervariable coding region (RHV) located between the conserved regions C2 and C3 in the amino acid sequence, two hypervariable coding regions are found in the *S-RNase* genes of *Solanaceae* and *Scrophulariaceae*. Further, only in the *S-RNase* genes of genus *Prunus* (family *Rosaceae*) are two introns present. The first intron is positioned between the signal peptide region and the C1 region of the mature protein, while the second intron (corresponding to the single intron of solanaceous and scrophulariaceous *S-RNase* genes) is placed in the hypervariable region. The presence of two introns variable in length in the *S-RNase* gene of genus *Prunus*, the basic p*I* of *S*-RNase proteins and their RNase activity were used for *S-RNase* allele detection.

In several plant families (including *Solanaceae* and *Rosaceae*) two groups of T2/S-type RNases, in addition to *S*-RNases, were detected and named acidic and basic ''non-*S* RNases'' according to their p*I* value. While the acidic ''non-*S* RNases'' were discovered to have roles in phosphate recycling and wounding response (Bariola et al., 1994; LeBrasseur et al., 2002), the function(s) of basic ''non-*S* RNases'' is(are) still unknown. The phylogenetic analysis of Igic and Kohn (2001) included 67 sequences of angiosperm T2/S RNases and related genes without RNase function, and revealed that acidic ''non-*S* RNases'' might have diverged before separation of monocots and dicots, showing that acidic ''non-*S* RNases'' are distantly related to *S*-RNases, while basic ''non-*S* RNases'' are more closely related to *S*-RNases.

The analysis presented in this paper was conducted on a wild almond, *Prunus webbii*, population from the Montenegrin region in the Balkans. *Prunus webbii* is an interesting species to study because of its possible contribution to the origin of almond. As a species that interbreeds with *Prunus dulcis* and one that is resistant to drought, it is also of interest for introgression in almond cultivars through breeding programs. As pointed out by Bošković et al. (2007), *Prunus webbii* was considered to be a self-compatible almond species at first, but later studies reported self-incompatible individuals in Italian and Spanish populations. Since *Prunus webbii* is SI displaying species, there are also questions regarding the origin of self-compatible individuals in *Prunus webbii*, i.e. whether identified SC alleles were introgressed in *Prunus webbii* from other *Prunus* species or they have genuinely arisen in *Prunus webbii*/its ancestor or both. For one self-compatible allele, *Sf*, found both in wild and cultivated almond, it has recently been revealed that it most likely originated in *Prunus dulcis* and was introduced in *Prunus webbii* afterwards (Bošković et al., 2007). Origins of other self-compatible wild almond alleles remain to be investigated.

To date, the *S*-RNases of *Prunus webbii* have been reported (http://www.ncbi.nlm.nih.gov/) for populations from Portugal, Australia, Spain and Italy, but this is the first record of *S*-RNases in wild almond populations in the Balkans. The data