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Characterization of one new non-S-RNase of *Armeniaca cathayana*

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Abstract: *Armeniaca cathayana*, a new species described in 2010, belongs to the gametophytic self-incompatibility (GSI) system which is under *S*-allele control. One new non-S-ribonuclease (non-S-RNase) was found in *A. cathayana* through comparing its nucleotide and amino acid sequences with sequences of the *S*-allele in the GenBank. The BLAST analysis showed that the one new non-S-RNase S68-RNase (GenBank Accession No. MH155952) had the highest 96% nucleotide sequence homology with *Prunus webbii* non-S-RNase PW_1 (EU809938.1). Alignment of deduced amino acid sequences of *A. cathayana* S68-RNase shared 83% similarity with *P. webbii* PW_1. The new non-S-RNase determined in this study will provide new information to GSI of Rosaceae.

Keywords: *Armeniaca cathayana*; gametophytic self-incompatibility; non-S-RNase homology

Armeniaca cathayana D.L. Fu, B.R. Li & J.M. Fu, a new species described in 2010, belongs to Rosaceae. It originates in Zhuolu, Hebei Province, China. *A. cathayana* seed has unique characteristics, such as large, flat sides, crisp and no bitter, which are different from *A. vulgaris* and *A. sibirica* (Fu *et al.* 2010). Meanwhile, Fu *et al.* (2011) verifies the taxonomic position of *A. cathayana* using SSR (simple sequence repeat) marker, which is consistent with the morphological classification results. The seed of *A. cathayana* has important economic value because of its high-quality and healthy oil with 95% of unsaturated fatty acids and more than 70% of monounsaturated fatty acids. Besides, it is also rich in proteins, minerals, vitamins, dietary fibers and trace elements required by the human body (Liu 2011).

The mechanism of *A. cathayana* fertilization belongs to gametophytic self-incompatibility (GSI) system which is under the control of *S*-allele. The product of the *S*-allele in styles is glycoprotein with ribonuclease activity, called S-ribonuclease (S-RNase). The *S*-allele encoding S-RNase is responsible for rejection of pollen grain which carries the same *S*-allele in the style (Xu *et al.* 2008). In addition to S-RNase, experimental findings have indicated that non-S-RNase genes and modifier genes are also included in GSI. Non-S-RNase genes are divided into two types, determining acidic and basic non-S-RNases (Ma & Oliveira 2000). The functions of some acidic non-S-RNases are involved in phosphate recycling in response to phosphate limitation, aging of tissue and wounding (LeBrasseur *et al.* 2002). However, the functions of basic non-S-RNases are not yet clear. Therefore, the data of the new non-S-RNase in this study will provide more complete insight into the GSI system of Rosaceae.

The amplificated profiles of 9 *A. cathayana* (ZL1, 80B05, 80D05, ZL8, ZL11, ZL12, ZL13, ZL16 and ZL18) using primers PF-h/PF-r (ATTTTCAATTTGTGCAACAATGG/CAAAAT ACCACTTCATGTAACAAC) are shown in Figure 1. All the bands were excised from gels and sequenced. The nucleotide

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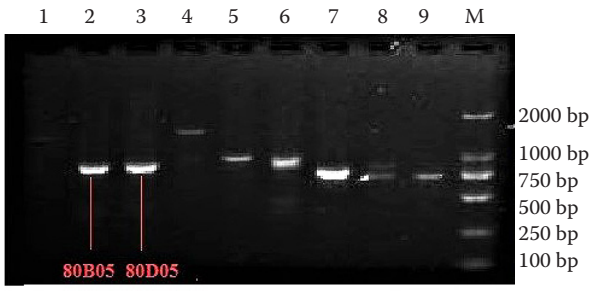

Figure1. The *S*-alleles electrophoretogram of 9 *Armeniaca cathayana* (1–9) using the primers PF-h/PF-r80B05 and 80D05 were provisional named S68-RNase

sequences were compared against the NCBI database, and the deduced protein sequences were then aligned to the known S-RNases. The results showed that one *S*-allele of 8 *A. cathayana* could be identified through BLAST analysis. The sequences of ZL08 were identified as S16 with a 1289 bp fragment, and the sequences of ZL11 and ZL12 were determined as S25 with a 925 bp fragment. Meanwhile, the ZL13, ZL16 and ZL18 had the S30 with a 662 bp fragment (Table 1 and Figure 1). However, the 80B05 and 80D05 amplified a 679 bp fragment using the primers PF-h/PF-r (provisional name S68-RNase). Through BLAST analysis, its nucleotide sequences were consistent with only two non-S-RNase genes. The highest similarity 96% was with *Prunus webbii* (wild almond) non-S-RNase gene PW_1 (EU809938.1) (Banović *et al*. 2009) with 100% query cover of nucleotide sequence (Figure 2). Alignment of deduced amino acid sequences for *A. cathayana* S68-RNase and *P. webbii* PW_1 gene was shown in Figure 3. BLAST analysis showed that the amino acid sequences of S68-RNase shared 83% similarity with *P. webbii* PW_1. There were five amino acid difference between *A. cathayana* S68-RNase and *P. webbii* PW_1 in RHV, a region of *S*-allele polymorphism. Lysine, methionine, serine and leucine in PW_1 were replaced with asparagine, lysine, asparagine and phenylalanine in S68-RNase,

Table 1. The *S*-allele identification of 9 *Armeniaca cathayana*

No.	Resources	*S*-allele	No.	Resources	*S*-allele
1	ZL01	–	6	ZL12	S25
2	80B05	S68-RNase	7	ZL13	S30
3	80D05	S68-RNase	8	ZL16	S30
4	ZL08	S16	9	ZL18	S30
5	ZL11	S25			

respectively. Meanwhile, one serine appeared in PW_1 is missing in S68-RNase. Moreover, the S68-RNase nucleotide sequence had a single intron with 60bp in length at nucleotide position 275. Second, S68-RNase showed 93% similarity with *P. avium* (sweet cherry) non-S-RNase (AB096918.1) (Yamane *et al*. 2003) with a lower query cover of 77% . Therefore,

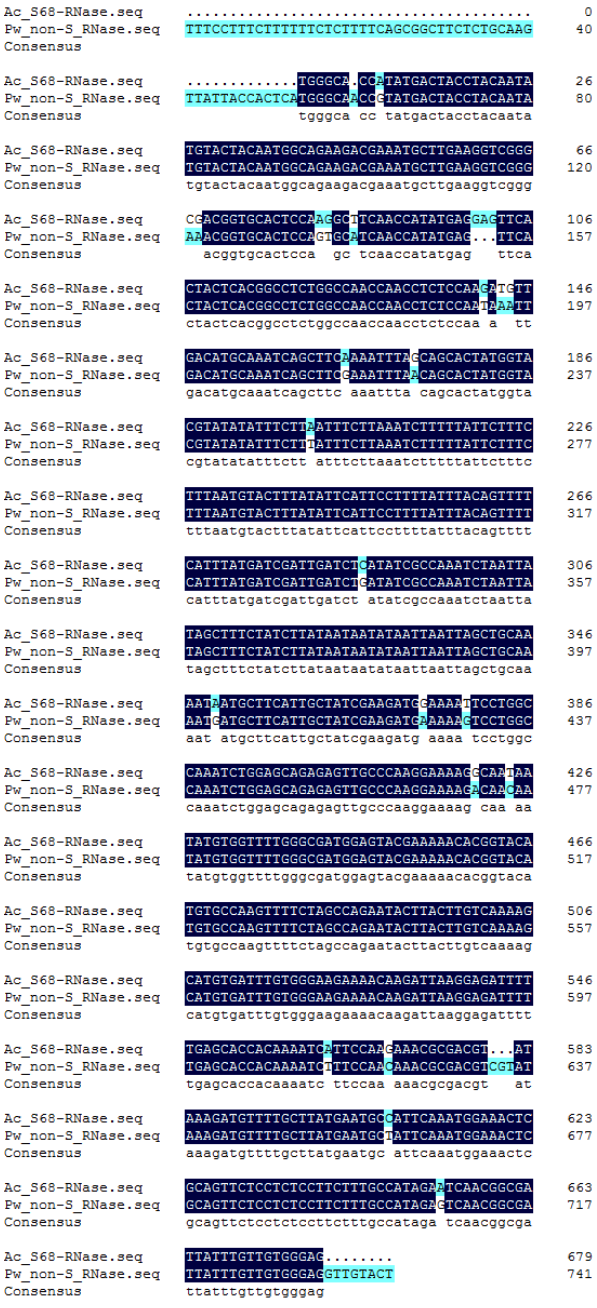

Figure 2. Nucleotide sequence comparison between *Armeniaca cathayana* non-S-RNase gene (S68-RNase) and *Prunus webbii* non-S-RNase gene (PW_1) (Ac as *Armeniaca cathayana;* Pw *as Prunus webbii*)

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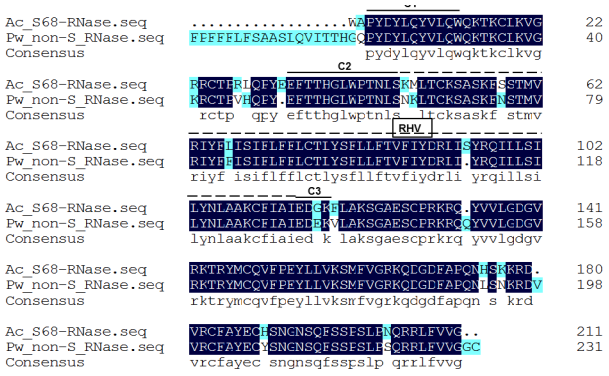

Figure 3. Alignment of deduced amino acid sequences for *Armeniaca cathayana* non-S-RNase gene (S68-RNase) and *Prunus webbii* non-S-RNase (PW_1) (Ac as *Armeniaca cathayana;* Pw *as Prunus webbii*)

the newly amplified nucleotide sequence differs from the NCBI database and was determined as the new non-S-RNase gene of *A. cathayana* (GenBank Accession No. MH155952).

Except for non-S-RNase of *A. cathayana*, several non-S-RNases had been reported in other species: RNase NS-1 in Japanese pear (*Pyrus pyrifolia*) (Norioka *et al*. 2007); RNase PW_1 in *P. webbii* (Banović *et al*. 2009); RNase NnSR1, non-S-RNases S5 and S63 in *Nicotiana alata* (Roldán *et al*. 2010; Rojas *et al*. 2013); PA1 and PA2 in sweet cherry (*P. avium*) (Yamane *et al*. 2003). The non-S-RNases were extremely similar to S-RNases in structure and specifically expressed in the style. Norioka *et al.* (2007) showed that the pear non-S-RNase was not only highly homologous to the tomato RNases LX and LE and the tobacco RNase NE, but also showed high similarity to the almond RNase PD1. Other Rosaceous stylar RNases, PD2 from almond and PA1 from sweet cherry, belong to a different subfamily than the pear non-S-RNases (Ma & Oliveira 2000; Yamane *et al*. 2003; Norioka *et al.* 2007). However, the physiological functions and origins of non-S-RNases were still not clear. Yamane *et al.* (2003) proposed a hypothesis based on the PA1 as an RNase in the characteristic phylogenetic position: basic non-S-RNases of *Prunus* could be an ancestral form of S-RNases in genus *Prunus*. Obviously, further sequences of basic non-S-RNases should be found to verify this hypothesis. If the hypothesis is valid, common ancestral RNase sequence of Rosaceae origin should be identified and characterised.

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