International Journal of
Molecular Sciences

MDPI

Article

Large-Scale Screening and Identification of *S-RNase* Alleles in Chinese and European Apricot Accessions Reveal Their Diversity and Geographic Distribution Patterns

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Abstract

Apricot (*Prunus armeniaca* L.) exhibits a gametophytic self-incompatibility (GSI) system. To identify the *S*-genotypes of the main apricot cultivars, including 133 native Chinese cultivars and 35 foreign accessions, PCR was performed using a combination of five primers based on the conserved regions of *Prunus S-RNase* genes. After cloning and sequencing the PCR products, the *S*-genotypes of all 168 apricot cultivars were determined. A total of 46 different *S-RNase* alleles, with 15 new alleles, were identified. For all 168 accessions, the top five most frequent *S-alleles* were S_8, S_{11}, S_9, S_{16}, and S_{53}. S_{11}, S_8, and S_{16} were the most frequent in Chinese cultivars, and S_9, S_8, and S_2 were mostly found in European accessions. For Chinese apricot cultivars, the distribution of *S-alleles* among five geographic regions was also investigated. In Northwest China, S_{16} was the most frequent *S-allele*. In the Xinjiang region, S_{66}, S_{49}, and S_{14} were the top three most frequent *S-alleles*. In North China, S_8, S_{11}, and S_{53} were the top three most frequent *S-alleles*. In addition, the self-compatible type, S_C, was not detected in these 133 Chinese accessions. Finally, the phylogenetic tree of apricot *S-alleles* indicated that there are four groups of *S-RNase* genes (S_{97}/S_{106}, $S_{14}/S_{14a}/S_{66}$, $S_9/S_{17}/S_{44}$, and S_{23}/S_{53}) presenting a very close relation. These results provide more data on the *S*-genotypes of apricot accessions, which can support future breeding programs by aiding in the selection of the appropriate parents and contributing to efficient orchard design by combining cultivars with suitable pollinizers.

Keywords: apricot; self-(in)compatibility; *S-alleles*; geographic distribution

Academic Editor: Jiangping Mao

Received: 17 July 2025
Revised: 28 August 2025
Accepted: 4 September 2025
Published: 5 September 2025

Citation: Zhang, J.; Zhang, M.; Yu, W.; Jiang, F.; Yang, L.; Ling, J.; Sun, H. Large-Scale Screening and Identification of *S-RNase* Alleles in Chinese and European Apricot Accessions Reveal Their Diversity and Geographic Distribution Patterns. *Int. J. Mol. Sci.* **2025**, *26*, 8667. https://doi.org/10.3390/ijms26178667

1. Introduction

Apricot (*Prunus armeniaca* L.), one of the most popular temperate tree fruit species, is widely grown around the world. The total planting area of apricot trees worldwide reached 5.73 million acres, with a total production of about 4.48 million tons (FAOSTAT, 2023, https://www.fao.org/faostat/ (accessed on 19 August 2025)). As the primary center of origin for apricot (*Prunus armeniaca* L.), China has extensive apricot germplasm diversity, with over 2000 distinct cultivars and landraces. The apricot fruit is attractive due to its unique pleasant aroma and high nutritional value. Unfortunately, most Chinese cultivars

exhibit self-incompatibility, resulting in low fruit setting [1]. In apricot production, the single plant yield has a significant positive correlation with the self-(in)compatibility of the cultivar [2]. Because of self-incompatibility, it is necessary to select and grow suitable pollinating trees when establishing an apricot orchard.

Traditional compatibility assessment in controlled pollination trials primarily relied on empirical knowledge derived from agricultural practice. Early diagnostic criteria classified cultivars as definitively self-compatible when exhibiting fruit set rates exceeding 6% through self-pollination [2,3]. However, the investigation of fruiting rates is time-consuming and labor-intensive. In the laboratory, fluorescence microscopy can be used to observe pollen tube growth, but this method requires expensive equipment and complex procedures. Advances in research have revealed that the self-incompatibility of apricots is gametophytic, controlled by a single *S*-locus with multiple alleles [4]. This locus includes at least the stigma *S-RNase* gene and the pollen *SFB* gene. When the pollen and stigma exhibit the same allele, the growth of the pollen tube in the stigma is hindered, resulting in self-incompatibility [5].

Studies have demonstrated that the *S-RNase* genes exhibit tissue-specific expression patterns in stigma tissues. *RNase* activity is crucial for the inhibition of pollen tube growth during the incompatibility response and may be involved in the degradation of ribosomal RNA [6,7]. Therefore, the *S*-genotype of a given cultivar can be quickly detected through specific PCR amplification of *S-alleles*, and the compatibility between any two cultivars can be determined by comparing their *S*-genotypes. This technology provides a scientific basis for the selection of pollen cultivars in production [8]. *S-allele* characterization has been successfully implemented across the main *Rosaceae* species, including apricot [9,10], plum [11,12], Japanese apricot [13], sweet cherry [14,15], almond [16], apple [17], and strawberry [18]. This methodology has also been extended to other self-incompatible fruit crops beyond Rosaceae, including citrus [19] and pomelo [20].

For apricot, extensive research has been conducted on the *S-RNase* genes of apricot worldwide since 1998. Burgos et al. used non-equilibrium pH gradient electrophoresis to separate and identify *S-RNases* associated with gametophytic self-incompatibility in nine apricot cultivars. This was the first study to report the *RNase* activities associated with the incompatibility alleles S_1, S_2, S_3, S_4, S_5, and S_6 and the compatibility *Sc* in apricot [4]. Subsequently, Romero cloned three *S-RNase* genes from the apricot genome [6]. In 2010, a total of 31 different *S*-genotypes were assigned to the 51 Turkish apricot cultivars. The *S-RNase* intron regions used to determine their lengths and the *S*-genotypes were detected via polymerase chain reaction (PCR) amplification [5]. The *S*-genotypes of 55 Moroccan apricot accessions were determined, resulting in 37 self-compatible genotypes [21]. The *S-alleles* of 44 new European apricot genotypes were further identified [22]. Boubakri et al. identified the *S*-genotypes of 68 Eurasian apricot variety groups from the Iran–Caucasus region and the Mediterranean basin planted in Tunisian regions. Self-compatible apricot cultivars were also discovered [23]. In contrast to Eurasian research, studies on Chinese apricot *S*-genotypes remain limited and have emerged more recently. In 2005, a pair of primers was designed, and *S-allele-specific* PCR was developed. Nine *S-alleles*, S_1–S_9, were first revealed via *S-allele*-specific PCR and confirmed via Southern blot analysis [24]. The *S*-genotypes of 16 apricot cultivars were also determined via the *S-allele* PCR approach, and the results were confirmed via cross-pollination tests among these cultivars [25]. Wu Jun et al. [26] analyzed the *S*-genotypes of 14 Chinese apricot cultivars and named eight new *S-alleles*. Jiang Xin et al. [27] detected the *S*-genotypes of 27 apricot varieties cultivated in Xinjiang and found 15 new *S-alleles*. Cumulatively, 96 apricot *S-RNase* genes have been registered in GenBank, reflecting both methodological progress and global collaboration.

However, despite the extensive documentation of apricot cultivars, research on their self-incompatibility (SI) systems remains disproportionately limited. Furthermore, the presence of synonymies and homonymies among some known *S-RNase* alleles led to a lack of comparability between different studies. Notably, identical cultivars have been assigned conflicting *S-RNase* genotypes in separate investigations. For instance, the *S-RNase* genotype of the same apricot cultivar 'Yinxiangbai' was reported as $S_{23}S_{36}$ by Wuyun et al. [28] vs. S_9S_{17} by Zhang et al. [25]. 'Honghebao' was also given two distinct *S-RNase* genotypes, S_9S_{11} and S_8S_9, in different studies [24,25], and the *S-RNase* genotype of 'Xinshiji' was recorded as S_7S_8 and S_9S_{10} in different studies. These discrepancies have limited the exchange of information. As a result, systematic research on the molecular mechanisms underlying apricot self-incompatibility has been hindered.

In China, apricot cultivation spans extensive areas across distinct geographical regions, primarily taking place in North China (Beijing, Tianjin, Hebei, Shanxi), Northwest China (Gansu, Shaanxi), and Northeast China (Liaoning, Heilongjiang, Jilin). There are also supplementary cultivation zones in Shandong and Henan. Regional cultivars show strong locality-specific characteristics, and there is some varietal overlap between regions, which contributes to remarkably diverse germplasm. However, most cultivars exhibit self-incompatibility, with *S*-genotype characterization remaining incomplete for many cultivars. Current *S*-genotype data for Chinese apricots remains limited, with fewer than 70 cultivars documented to date [24–26]. This incomplete understanding of incompatibility relationships has impeded the use of parental selection in hybrid breeding programs and the configuration of effective pollination trees in commercial orchards.

In this study, the *S-RNase* genotypes of 168 apricot cultivars, primarily native to China, were determined through targeted PCR analysis and *S-RNase* sequencing. Then, the *S-allele* frequency distribution patterns in Chinese apricot accessions were compared to those in foreign ones, and the geographic distribution of *S-allele* frequencies within Chinese apricot cultivars was analyzed. Furthermore, we performed molecular characterization of self-compatibility determinants in selected high-yield genotypes, aiming to identify S_C alleles within Chinese apricot accessions. The findings aid in establishing cross-incompatibility groups to avoid pollination problems in orchards and provide useful information for breeders in selecting parental genotypes. The novel *S-RNase* allele sequences obtained in this study provide critical data resources for advancing phylogenetic analyses of *S*-locus evolution within *Rosaceae* species.

2. Results

2.1. Identification of S-Alleles in Apricot

The *S*-genotypes of 168 apricot cultivars were characterized through PCR amplification of the second intron regions using five primer pairs, followed by sequencing and homology analysis with DNAMAN 8 software. Sequence alignment revealed 99–100% similarity among fragments of identical/near-identical length. Exon-derived amino acid sequences demonstrated complete conservation (100% homology) across all samples. Comprehensive analysis combining intron size polymorphisms with sequence patterns identified 46 distinct *S-alleles* through NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 20 February 2025))verification. Thirty-one alleles matched previously reported apricot *S-RNase* genes, while fifteen represented novel *S-alleles*. The fragment sizes and the GenBank accession numbers are shown in Table 1. The second introns of all 31 *S-RNase* genes were found within the hypervariable region (RHV), with sizes ranging from 180 bp (S_{10}) to 1749 bp (S_{20}), demonstrating a high degree of length polymorphism that distinguishes the different *S-RNase* alleles.

Table 1. Sequence from 168 apricot cultivars aligned with 31 published *S-alleles*.

S-Allele	PCR Fragment Size (bp)/Intron Sizes (bp)					Genebank Accession No.
	EM-PC2consFD, EM-PC3consRD	Pru-C2, Amy-C5	Pru-C2, PCE-R	ASI II, Amy-C5	PaCons II-F, PaCons II-R	
S_2	895/706	1096/706				AY587562.1
S_8		827/410	573/409	928/411		AY884212.1
S_9		885/467				AY853594.1
S_{10}	266/180					AY846872.1
S_{11}	464/275	672/275				DQ868316.1
S_{12}	359/171					DQ870628.1
S_{13}	401/212					DQ870629.1
S_{14}	493/305					DQ870630.1
S_{14a}	495/309					GU574199.1
S_{15}	469/283					DQ870631.1
S_{16}	481/292	700/292				DQ870631.1
S_{17}	657/461					DQ270001.1
$S_{18\text{-}1}$ *	307/108					DQ270000.1
$S_{18\text{-}2}$ *	1337/1148	1546/1148				DQ870634.1
S_{20}	1936/1749					EF160078.1
S_{23}	693/505					EU037262.1
S_{24}	357/168	588/168				EU037263.1
S_{25}	772/583	994/584				EU037264.1
S_{26}			416/289			EU037265.1
S_{28}		1352/946				EU836684.1
S_{30}		726/285				EF185301.1
S_{35}	312/124					GU574196.1
S_{36}		718/299				GU574198.1
$S_{40\text{-}1}$ *	539/353	749/353				GU354239.1
$S_{40\text{-}2}$ *				542/164		HQ342870.1
S_{44}			635/464			HQ342874.1
S_{49}		653/212				HQ342879.1
S_{52}	1296/1111	1512/1110				KF951503.2
S_{53}		965/508				KF975455.2
S_{54}					1296/891	KT223013.1
S_{66}		704/308				JQ317152.1

* $S_{18\text{-}1}$ and $S_{18\text{-}2}$, represent two different S_{18} alleles with different Genebank accession No.; $S_{40\text{-}1}$ and $S_{40\text{-}2}$ represent two different S_{40} alleles with different Genebank accession No.

Most alleles showed unique GenBank accession correspondence. However, some *S-RNase* genes, such as S_{18}, S_{40}, and S_{52}, have the same *S-allele* accession number that corresponds to two GeneBank accession numbers. Furthermore, the gene sequences and amino acid sequences are also completely different. For S_{52}, after BLAST searches in the NCBI database, the sequence of the *A*-S_{52} allele was matched to two *Pa*S_{52}-RNases from two different apricot cultivars, 'Daguohuanna' and 'Kabakehuanna', under the accession numbers KF951503.2 and HQ342882.1, respectively. KF951503.2 showed the complete sequence of S_{52}, while HQ342882.1 was a partial sequence. In this study, the amino acid (AA) sequences from seven different cultivars with A-S_{52} showed the highest similarity to S_{52}, with the accession number KF951503.2. It is different from the pattern of S_{52}, and there were four cultivars that were assigned S_{18}, associated with different Genebank accession Numbers: (DQ270000.1 and DQ870634.1) https://www.ncbi.nlm.nih.gov/nuccore/DQ270000.1/, https://www.ncbi.nlm.nih.gov/nuccore/DQ870634.1 (accessed on 20 February 2025)) are marked as $S_{18\text{-}1}$ and $S_{18\text{-}2}$ in this study. $S_{40\text{-}1}$ and $S_{40\text{-}2}$ represent two different S_{40} with different Genebank accession Nos. (GU354239.1 and HQ342870.1) (https://www.ncbi.nlm.nih.gov/nuccore/GU354239.1, https://www.ncbi.nlm.nih.gov/nuccore/HQ342870.1 (accessed on 20 February 2025)).

2.2. Identification of New S-Alleles in Apricot

The DNA sequences were initially aligned with the *Prunus S-RNase* cDNA sequence exhibiting the highest homology from GenBank. Intron/exon boundaries were identified using the conserved GT/AG splicing rule, with intron sizes determined subsequently. Corresponding amino acid sequences were deduced through DNAMAN software analysis and compared against existing *S-RNase* homologs in GenBank. Fifteen novel *S-alleles* were identified with characteristic *Prunus S-RNase* structural features, including four conserved domains (C2, C3, RC4, and C5), a hypervariable region (RHV), and an undocumented, unique intron size configuration. Critical analysis revealed distinct RHV variations in these sequences compared to all registered *P. armeniaca S-RNase* genes in GenBank. Based on sequence homology analysis through NCBI GenBank and following the nomenclature protocols of Vilanova et al. [29] and Halász et al. [5], these new alleles were designated as S_{93}–S_{107}, continuing the existing *S-RNase* gene number in GenBank. The novel sequences have been deposited in GenBank under the accession numbers PV206780–PV206794, with specific assignments corresponding to each allele (Table 2). The second introns within the RHV demonstrated significant length polymorphism (90 bp -1214 bp), providing distinctive molecular characters for different *S-alleles* (Figure 1 and Table 2).

Table 2. Fifteen new *S-alleles* in apricot accessions.

S-Alleles	Cultivar No.	Cultivar Name	Primer Pairs and PCR Fragment Size (bp)/Intron Sizes (bp)		GeneBank Accession No.
			EM-PC2consFD, EM-PC3consRD	Pru-C2, Amy-C5	
S_{93}	22	Jingjia No. 2	273/90		PV206781
S_{94}	46	Dafeng	502/319		PV206782
S_{95}	28	Jingren No.4		683/275	PV206783
S_{96}	74	Hongjinzhen		575/173	PV206784
S_{97}	58	Xingtaihongjiexing		746/458	PV206785
S_{98}	25	Jingren No.1		871/460	PV206791
S_{99}	158	Harmat		884/460	PV206786
S_{100}	82	Dongning No.2		924/522	PV206792
S_{101}	96	Lintonghongxing		1348/928	PV206787
S_{102}	23	Jingluofeng		1416/996	PV206793
S_{103}	52	Longwangmao		1452/1044	PV206788
S_{104}	75	Jinkaite		1466/1035	PV206789
S_{105}	100	Niujiaobangzi		1625/1214	PV206794
S_{106}	31	Longquanwuxiangbai	533/346		PV206790
S_{107}	69	Yuhankui	1227/1046		PV206780

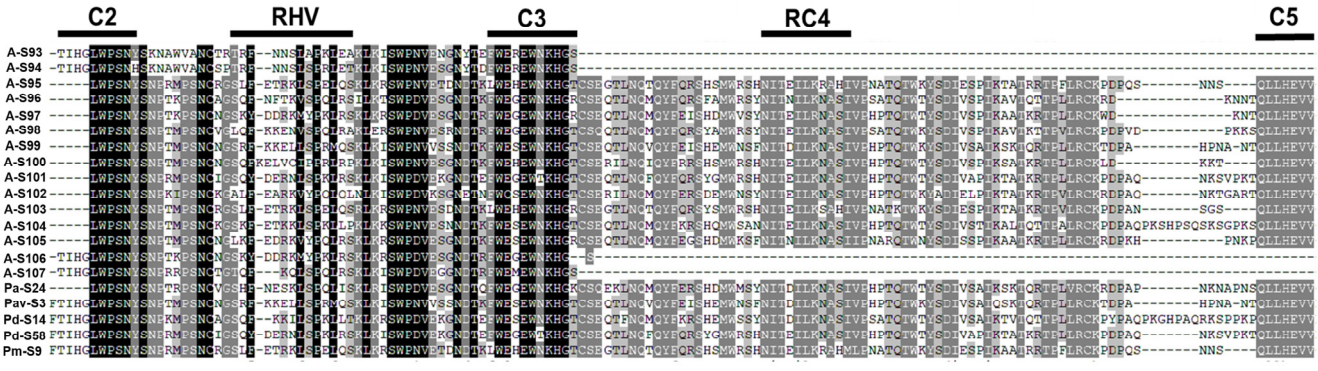

Figure 1. Alignment of the deduced amino acid sequences of *S-RNase* from apricot and other *Prunus* species. Four conserved regions, C2, C3, RC4 and C5, were marked with rectangles, and one

hypervariable region, RHV, was underlined. A-: *S-RNases* of apricot (*P. armeniaca*) were detected in this study; Pa-: *S-RNases* of apricot (*P. armeniaca*) have been published; Pav-: *P. avium*; Pd-: *P. dulcis*; Pm-: *P. mume*. GenBank accession numbers of *Prunus* species: *Pa-S*$_{24}$: ABS84176.1; *Pav-S*$_3$: AAT72119.1; *Pd-S*$_{14}$: CAJ77745.1; *Pd-S*$_{58}$: CBI68346.1; *Pm-S*$_9$: BAF91157.1. The different colors represent the conserved percentage among sequences: black, 100%; darkgrey, 80%; grey, 60%.

2.3. Identification of Sc-Allele

Previous studies have confirmed that the coding regions of S_8- and S_C-RNase alleles are identical, with the S_8- and S_C-haplotypes differing exclusively in their SFB gene structure. Specifically, a 358 bp insertion was identified in the SFB_C. To discriminate the S_C-haplotype, we implemented a two-tiered molecular strategy. Initially, the allele-specific primer pair AprSC8R/PaConsI F was selected to amplify the S_C/S_8-RNase allele. As demonstrated in Figure 2A, a 546 bp fragment was successfully amplified in six cultivars, including the positive control cultivars 'Bergeron' (S_2S_C) and 'Bora' (S_9S_C), with determined *S*-genotypes [30]. In contrast, no amplification products were observed in the negative control cultivar 'Hargrand' (S_1S_2). Subsequently, employing the primer pair AprFBC8 [5], we distinguished between the S_8- and S_C-alleles. Cultivars carrying the SFB_C-allele showed an amplification product fragment of approximately 500 bp, whereas those with the SFB_8-allele produced a fragment of about 150 bp (Figure 2B). The combinatorial results conclusively revealed that only three cultivars, 'Nifa', 'Bora', and 'Bergeron', were self-compatible, carrying the S_C-haplotype. The S_8-allele was identified in 'H-48', 'Zhupishui', and '99-31' germplasm (Figure 2B).

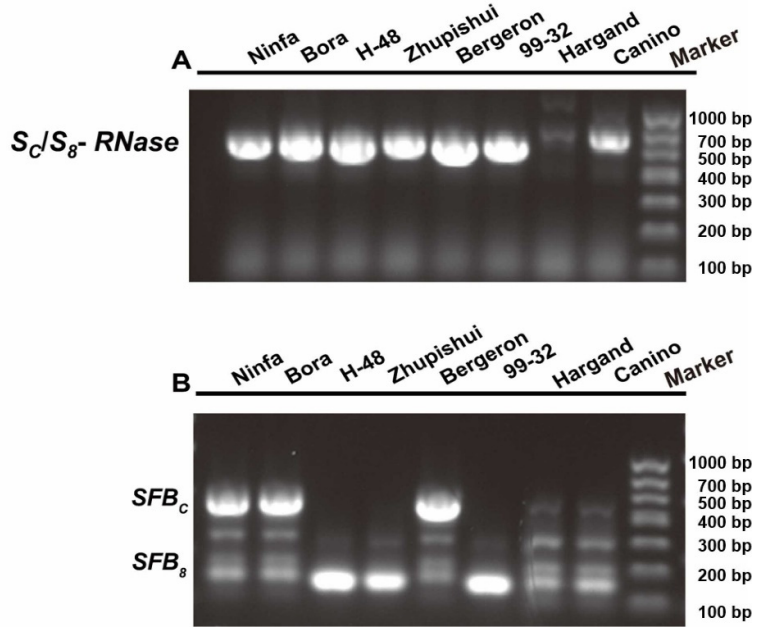

Figure 2. PCR detection and reliable differentiation of S_C- and S_8-haplotypes in eight apricots. (**A**) PCR used to identify selectively the S_8/S_C-RNase alleles. (**B**) Amplification of the *SFB* gene used to differentiate between SFB_C and SFB_8 alleles [M = 1 kb + DNA ladder].

2.4. Analysis of S-Genotypes of 168 Apricot Cultivars

Table 3 presents the *S*-genotype profiles of 168 apricot cultivars collected from diverse geographical regions. Among these cultivars, 122 (72.6%) accessions exhibited heterozygous *S*-genotypes at their *S-RNase* loci, demonstrating two distinct *S-alleles* for each cultivar. The other 46 cultivars, such as 'Dabada', 'Fangshanhongxing', 'Guanyelian', 'Liquanerzhuanzi', 'Jingren No.1', 'Zhupishui', 'Daxingmei', and '99-45', showed mono-allelic expression at the *S*-locus, with only one detectable *S-allele*, while the complementary allele

remained unidentified. Notably, 31 cultivars were found to carry novel *S-RNase* alleles (S_{93}–S_{107}) that were previously uncharacterized.

Table 3. *S*-genotypes of 168 apricot cultivars.

No.	Cultivar	Province, Country of Origin	S-Genotype	Areas
1	Baixing 10-38	Beijing, China	S_9S_{10}	
2	Beianhe	Beijing, China	S_9S_{17}	
3	Beishandabian	Beijing, China	$S_{10}S_{53}$	
4	Beizhaihongxing	Beijing, China	$S_{93}S_{103}$	
5	Chuanling	Beijing, China	S_8S_{53}	
6	Dabada	Beijing, China	S_{36}	
7	Fangshanhongxing	Beijing, China	S_{11}	
8	Fangshanxiangbai	Beijing, China	$S_{17}S_{53}$	
9	Guajiayutianhexiangbai	Beijing, China	S_8S_{17}	
10	H20-5	Beijing, China	S_9S_{95}	
11	H21-25	Beijing, China	$S_{23}S_{53}$	
12	H23-37	Beijing, China	$S_{14}S_{66}$	
13	H23-43	Beijing, China	$S_{14}S_{66}$	
14	H23-44	Beijing, China	$S_{14}S_{66}$	
15	H-48	Beijing, China	S_8S_{52}	
16	Honghuomeizi	Beijing, China	S_8S_{53}	
17	Huangjianzui	Beijing, China	S_2S_{66}	
18	Jingcuihong	Beijing, China	$S_{10}S_{11}$	
19	Jingfeihong	Beijing, China	S_8S_{11}	
20	P35-146	Beijing, China	S_8S_{30}	
21	Jingjia No.1	Beijing, China	$S_{24}S_{49}$	
22	Jingjia No.2	Beijing, China	$S_{24}S_{93}$	
23	Jingluofeng	Beijing, China	$S_{11}S_{102}$	
24	Jingluohong	Beijing, China	S_8S_{95}	
25	Jingren No.1	Beijing, China	S_{98}	
26	Jingren No.2	Beijing, China	S_8	
27	Jingren No.3	Beijing, China	S_{103}	North China
28	Jingren No.4	Beijing, China	S_{95}	
29	Jingren No.5	Beijing, China	S_{24}	
30	Jingxianghong	Beijing, China	$S_{10}S_{11}$	
31	Jingzaohong	Beijing, China	S_9S_{36}	
31	Longquanwuxiangbai	Beijing, China	$S_{53}S_{106}$	
33	Luotuohuang	Beijing, China	S_8S_{11}	
34	Mituoluo	Beijing, China	S_{11}	
35	P51-54	Beijing, China	$S_{11}S_{17}$	
36	Pingguohong	Beijing, China	S_8S_{66}	
37	Shanbaixing	Beijing, China	$S_{17}S_{53}$	
38	Shanhuangxing	Beijing, China	S_8S_{11}	
39	Xiaoyubada	Beijing, China	$S_{23}S_{53}$	
40	Yingchun	Beijing, China	$S_{24}S_{36}$	
41	Zaoxiangbai	Beijing, China	$S_{10}S_{53}$	
42	Zhuyaozi	Beijing, China	$S_{10}S_{35}$	
43	Guanlaoyelian	Tianjin province, China	S_8S_{16}	
44	Wanxiangbai	Tianjin province, China	$S_{17}S_{53}$	
45	Cangzaotian No.1	Hebei province, China	$S_{11}S_{49}$	
46	Chuanzhihong	Hebei province, China	S_8S_{24}	
46	Dafeng	Hebei province, China	S_8S_{94}	
48	Erhongxing	Hebei province, China	$S_{11}S_{17}$	
49	Ganyu	Hebei province, China	S_8S_{16}	
50	Jiguang	Hebei province, China	S_8S_9	
51	Jinyu	Hebei province, China	$S_{13}S_{52}$	

Table 3. *Cont.*

No.	Cultivar	Province, Country of Origin	*S*-Genotype	Areas
52	Longwangmao	Hebei province, China	$S_{11}S_{103}$	
53	Muguaxing	Hebei province, China	$S_{11}S_{16}$	
54	Qingmisha	Hebei province, China	$S_{9}S_{44}$	
55	Shizixing	Hebei province, China	$S_{10}S_{53}$	
56	Tianedan	Hebei province, China	$S_{9}S_{16}$	
57	Xingtaidahongxing	Hebei province, China	$S_{17}S_{36}$	
58	Xingtaihongjiexing	Hebei province, China	$S_{8}S_{97}$	
59	You No.1	Hebei province, China	$S_{11}S_{103}$	
60	You No.2	Hebei province, China	S_{11}	
61	Zaohongxing	Hebei province, China	S_{36}	
62	Zaohuang	Hebei province, China	$S_{9}S_{53}$	
63	Guanyelian	Shanxi province, China	S_{28}	
64	Hongbada	Henan province, China	S_{101}	
65	Lixing	Henan province, China	$S_{40\text{-}1}$	
66	Mixiangxing	Henan province, China	$S_{11}S_{15}$	
67	Yangshaohuang No.1	Henan province, China	$S_{13}S_{102}$	Central China
68	Yangshaohuang No.2	Henan province, China	$S_{36}S_{102}$	
69	Yuhankui	Henan province, China	$S_{11}S_{107}$	
70	Yuzaoguan	Henan province, China	$S_{9}S_{53}$	
71	Badou	Anhui, China	$S_{36}S_{102}$	
72	Caizihuang	Shandong province, China	$S_{11}S_{16}$	
73	Honghebao	Shandong province, China	$S_{9}S_{16}$	
74	Hongjinzhen	Shandong province, China	S_{96}	
75	Jinkaite	Shandong province, China	$S_{11}S_{104}$	East China
76	Kuijin	Shandong province, China	$S_{11}S_{102}$	
77	Laoshanhong	Shandong province, China	S_{11}	
78	Pingdingzhen	Shandong province, China	$S_{12}S_{36}$	
79	Qingdaodahong	Shandong province, China	S_{11}	
80	Zaoyu	Shandong province, China	S_{20}	
81	Dongning No.1	Heilongjiang province, China	$S_{16}S_{100}$	
82	Dongning No.2	Heilongjiang province, China	S_{100}	Northeast China
83	Baixing	Liaoning province, China	S_{10}	
84	Daxingmei	Liaoning province, China	S_{8}	
85	Guofeng	Liaoning province, China	$S_{8}S_{18\text{-}2}$	
86	Caoxing	Gansu province, China	$S_{8}S_{17}$	
87	Dajiexing	Gansu province, China	S_{16}	
88	Dapiantou	Gansu province, China	$S_{36}S_{102}$	
89	Zhupishui	Gansu province, China	S_{8}	
90	Taoxing	Ningxia province, China	S_{16}	
91	Meixing	Qinghai province, China	S_{25}	
92	Caopixing	Shaanxi province, China	S_{16}	
93	Haidongxing	Shaanxi province, China	S_{16}	
94	Jidanxing	Shaanxi province, China	$S_{25}S_{26}$	Northwest China
95	Lanzhuhong	Shaanxi province, China	$S_{16}S_{23}$	
96	Lingtonghongxing	Shaanxi province, China	S_{101}	
97	Lintonghongxing No.2	Shaanxi province, China	S_{16}	
98	Liquanerzhuanzi	Shaanxi province, China	S_{16}	
99	Machuanling	Shaanxi province, China	$S_{11}S_{16}$	
100	Niujiaobangzi	Shaanxi province, China	S_{105}	
101	Niujiaohuang	Shaanxi province, China	S_{105}	
102	Qinwang	Shaanxi province, China	$S_{40\text{-}2}$	
103	Touwojie	Shaanxi province, China	S_{16}	
104	Xinong 25	Shaanxi province, China	$S_{10}S_{36}$	

Table 3. *Cont.*

No.	Cultivar	Province, Country of Origin	*S*-Genotype	Areas
105	Yinxiangbai	Shaanxi province, China	$S_{36}S_{53}$	
106	Zaotianhe	Shaanxi province, China	$S_{16}S_{105}$	
107	Zhanggongyuan	Shaanxi province, China	$S_{24}S_{25}$	
108	Ake	Xinjiang, China	$S_{12}S_{66}$	
109	Chibangzi	Xinjiang, China	$S_{13}S_{49}$	
110	Cuijianali	Xinjiang, China	$S_{49}S_{66}$	
111	Dabaiyou	Xinjiang, China	$S_{18\text{-}2}S_{49}$	
112	Daguohuanna	Xinjiang, China	$S_{14}S_{49}$	
113	Dayoujia	Xinjiang, China	$S_{49}S_{66}$	
114	Heiyexing	Xinjiang, China	$S_{16}S_{66}$	
115	Kezimayisang	Xinjiang, China	$S_{14a}S_{66}$	
116	Kuikepiman	Xinjiang, China	$S_{11}S_{53}$	
117	Kumaiti	Xinjiang, China	$S_{49}S_{66}$	
118	Liguangxing	Xinjiang, China	$S_{24}S_{49}$	
119	Muyage	Xinjiang, China	$S_{14}S_{66}$	
120	Pinaizi	Xinjiang, China	$S_{13}S_{49}$	
121	Qiaoerpang	Xinjiang, China	$S_{14}S_{66}$	
122	Saimaiti	Xinjiang, China	$S_{24}S_{53}$	
123	Shushangganxing	Xinjiang, China	$S_{14}S_{66}$	
124	Xinjiangshaxing	Xinjiang, China	$S_{8}S_{102}$	
125	Xinshisheng	Xinjiang, China	$S_{52}S_{53}$	
126	Bingtangwei	China	$S_{17}S_{25}$	Unclear
127	Haihongzhen	China	$S_{9}S_{17}$	
128	Haiquanhong	China	$S_{8}S_{11}$	
129	Hongxing	China	S_{11}	
130	Kuhehonglian	China	$S_{16}S_{102}$	
131	Longjingbaixing	China	$S_{15}S_{16}$	
131	Xiaopuxiangbai	China	$S_{17}S_{53}$	
133	Yinxing	China	$S_{23}S_{53}$	
134	Meiwuming	American	$S_{2}S_{8}$	Foreign areas
135	99-2	Czech Republic	$S_{8}S_{9}$	
136	99-12	Czech Republic	S_{24}	
137	99-15	Czech Republic	$S_{8}S_{9}$	
138	99-27	Czech Republic	S_{52}	
139	99-31	Czech Republic	$S_{8}S_{66}$	
140	99-37	Czech Republic	$S_{17}S_{18\text{-}2}$	
141	99-38	Czech Republic	S_{11}	
142	99-43	Czech Republic	$S_{9}S_{17}$	
143	99-44	Czech Republic	$S_{24}S_{9}$	
144	99-45	Czech Republic	S_{11}	
145	Aurora	Czech Republic	$S_{8}S_{9}$	
146	Betinka	Czech Republic	$S_{8}S_{52}$	
147	Hargand	Czech Republic	S_{2}	
148	Jennycot	Czech Republic	$S_{2}S_{9}$	
149	Jitka	Czech Republic	$S_{24}S_{49}$	
150	LE5137	Czech Republic	S_{24}	
151	Rumjanaja	Czech Republic	$S_{8}S_{53}$	
152	Bergeron	France	$S_{2}S_{C}$	
153	Canino	France	$S_{2}S_{9}$	
154	Early orange	France	$S_{9}S_{11}$	
155	Cegledi bibor kajszi	Hungary	$S_{14}S_{66}$	
156	Cegledi orias	Hungary	$S_{14}S_{66}$	
157	Cegledi piroska	Hungary	S_{36}	
158	Harmat	Hungary	S_{99}	

Table 3. *Cont.*

No.	Cultivar	Province, Country of Origin	S-Genotype	Areas
159	B088	Italy	S_{54}	
160	B089	Italy	S_2S_9	
161	B095	Italy	$S_{49}S_{93}$	
162	Bora	Italy	S_9S_C	
163	Corlate	Italy	$S_{18\text{-}1}$	
164	Ninfa	Italy	S_2S_C	
165	Wondercot	Italy	S_9S_{52}	
166	Yidalixing	Italy	S_{52}	
167	Pinghexing	Japan	S_8S_9	
168	Xinzhoudashi	Japan	S_{16}	

These results were supported by a previous study on controlled cross-pollination tests for some apricot cultivars [1]. The fruit set percentages of 'luotuohuang' × 'Honghebao', 'luotuohuang' × 'Dapiantou', 'Dapiantou' × 'Honghebao', 'Xinong 25' × 'Luotuohuang' were 10.8–16.7%. According to the accepted criteria [2,3], these cultivars are cross-compatible. Correspondingly, in this study, each combination of cultivars has a different *S*-genotype. The S-genotypes of 'luotuohuang', 'Honghebao', 'Dapiantou', and 'Xinong 25' were S_8S_{11}, S_9S_{16}, $S_{36}S_{102}$, and $S_{10}S_{36}$, respectively. 'Chuanling' (S_8S_{53}) and 'Luotuohuang' (S_8S_{11}), which shared one *S-allele*, were considered as semi-compatible and, usually, cannot be selected as pollinizers for each other.

2.5. S-Allele Frequency Distribution Patterns Between Chinese and Foreign Apricot Accessions

As illustrated in Figure 3, S_8 emerged as the predominant *S-allele* across all 168 apricot accessions, followed sequentially by S_{11}, S_9, S_{16}, and S_{53}. Comparative analysis revealed distinct distribution patterns between Chinese cultivars and foreign accessions. For Chinese apricot cultivars, S_{11} was the most frequent *S-allele* (occurred in 26 genotypes), followed by S_8 (in 23 genotypes), S_{16} (in 20 genotypes), S_{53} (in 19 genotypes), S_{66} (in 14 genotypes), S_{17} (in 12 genotypes), S_9 (in 11 genotypes), and S_{49} (in 10 genotypes). The remaining 35 *S-alleles* occurred at relatively lower frequencies, each present in fewer than 10 genotypes. For S_2, S_{14a}, S_{20}, S_{26}, S_{28}, S_{30}, $S_{40\text{-}1}$, $S_{40\text{-}2}$, S_{44}, S_{94}, S_{96}, S_{97}, S_{98} S_{104}, S_{106}, and S_{107}, each *S-allele* was detected in only one genotype. For foreign accessions, S_9, S_8, S_2, S_{24}, and S_{52} were the top three most frequent, occurring in 12, 8, and 7 genotypes, respectively. Each of the eight *S-alleles*, including S_{16}, $S_{18\text{-}1}$, $S_{18\text{-}2}$, S_{36}, S_{53}, S_{54}, S_{93}, and S_{99}, was also detected in only one European cultivar. In both Chinese apricot cultivars and foreign accessions, S_8 and S_9 were the relatively more frequent *S-alleles*. *Sc* was found in only three European genotypes, and could not be detected in the tested Chinese apricot cultivars. In addition, it was found that S_{53} was mostly found in white-fleshed apricot cultivars, such as 'Xiaoyubada', 'Fangshanxiangbai', 'Shanbaixing', 'Chuanling', and 'Zaoxiangbai', which comprised 11 of the 19 cultivars with S_{53} (Table S1).

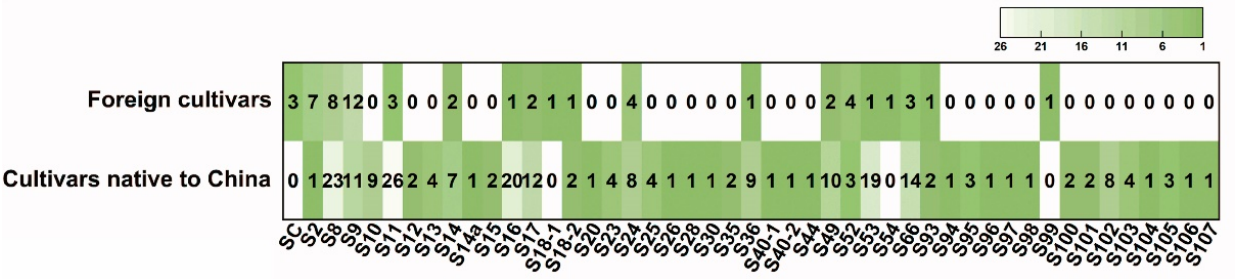

Figure 3. *S-allele* frequency distribution of Chinese apricots and foreign accessions.

2.6. Geographic Distribution Patterns of S-Allele Frequencies in Chinese Apricot Cultivars

The distribution of *S-alleles* demonstrated significant geographic dependency among Chinese apricot accessions, with distinct frequency patterns emerging across five major regions (Figure 4 and Table S2). The key distribution characteristics of the alleles exhibit a diverse geographic spectrum. There are three alleles, S_{11}, S_{16}, and S_{102}, that are present in four regions. Additionally, there are tri-regional alleles, such as S_8, S_9, S_{10}, S_{13}, S_{18}, S_{36}, and S_{53}; bi-regional alleles, like S_{14} S_{17}, S_{23}, S_{24}, S_{35}, S_{40}, S_{49}, S_{52}, S_{66}, and S_{101}; and 25 alleles that are specific to single regions. In terms of regional frequency profiles, the northwestern region of China, encompassing Gansu, Shaanxi, Ningxia, and Qinghai, has 40 accessions containing 23 *S-alleles*, with S_{16} being the dominant allele. The Xinjiang region has 18 accessions with 14 alleles, with S_{66} leading in frequency at 25%, followed by S_{49} at 22%, and S_{14} at 11% (Table 3). In North China, which includes Beijing, Tianjin, Hebei, and Shanxi, there are 63 accessions exhibiting 28 alleles. The most frequent alleles are S_8 at 15%, S_{11} at 13%, S_{53} at 10%, and S_9 and S_{17} at 7%. In the northeastern part of China, there are five cultivars with five *S-alleles* (S_8, S_{10}, S_{16}, $S_{18\text{-}2}$, and S_{100}) detected, with S_8 and S_{100} being the dominant *S-alleles*. In Central China, specifically Henan Province, there are seven accessions containing 11 alleles, with S_{11} and S_{102} co-dominant at 17% each. Lastly, in East China, which covers Shandong and Anhui, there are ten accessions with nine alleles, with S_{11} being the predominant allele at 31%.

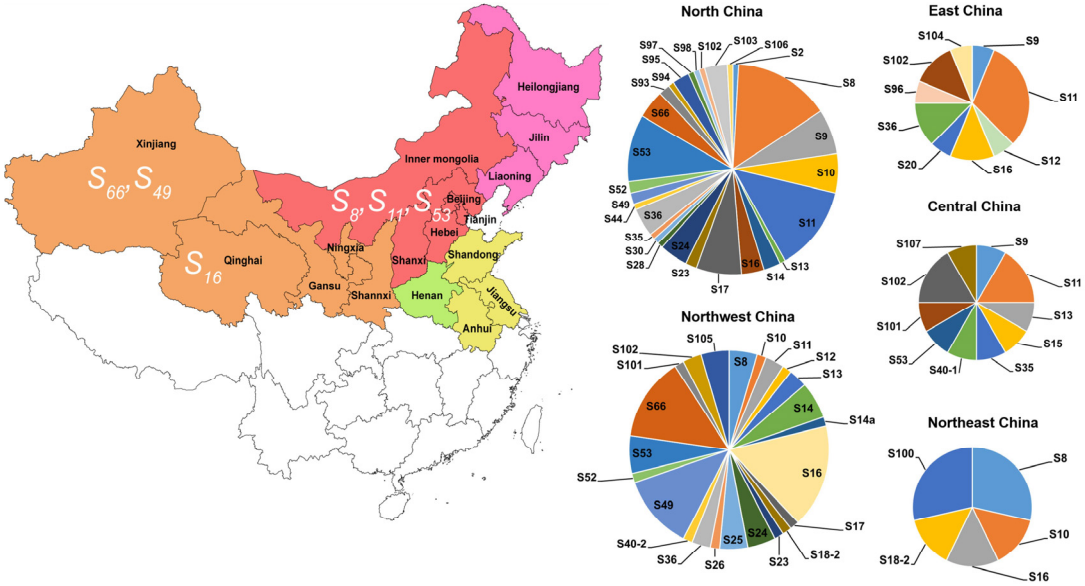

Figure 4. *S-allele* frequency distribution according to geographic areas in China. A map of the five major production areas of apricots in China: northwest area (orange color), North China (pink color), northeast area (red color), Central China (green color), and East China (yellow color). Relative frequencies for *S-alleles* (pie charts) are shown for each area.

2.7. S-RNase Gene Sequence Alignment and Phylogeny

The predicted amino acid sequences between the C2 and C5 regions from 46 detected *S-RNases* were aligned with each other using the Clustal W algorithm, and a neighbor-joining tree was constructed. The phylogenetic tree demonstrated that there are four groups of *S-RNase* genes that are closely related, such as S_{97} and S_{106}; S_{14}, S_{14a}, and S_{66}; S_9, S_{17}, and S_{44}; and S_{23} and S_{53} (Figure 5). Detailed sequence comparisons showed distinct amino acid (AA) variations among these groups. For S_{97} and S_{106}, a single amino acid difference was observed in the C3 domain. S_{14a} vs. S_{14}, displayed two AA and four AAs variations in the C2 and C3 conserved regions, respectively. Both S_{14} and S_{14a} exhibited an additional valine

residue (V) in the hypervariable region (RHV) compared to S_{66}. Compared to S_9, S_{17} lacked one valine residue (V) in the RHV, while S_{44} lacked two amino acid residues (leucine and valine, L and V). In comparison with S_{53}, S_{23} has a deletion of one amino acid (a tyrosine, Y) in the RHV region and has another difference of one amino acid in the C3 region.

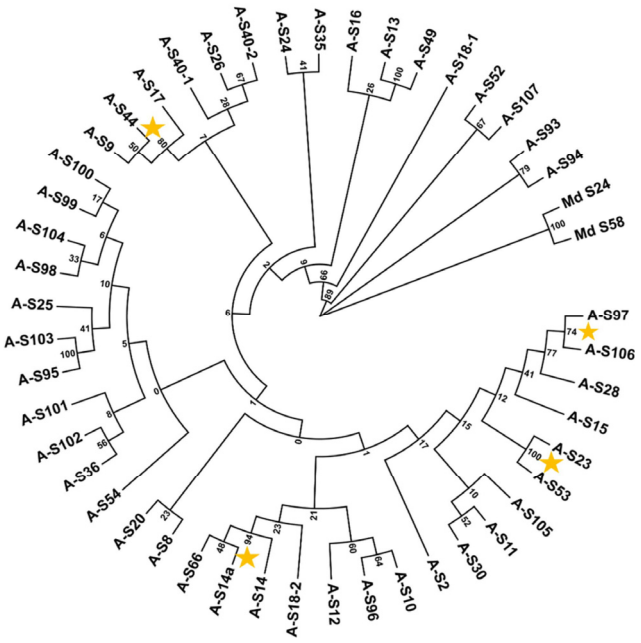

Figure 5. Phylogram depicting evolutionary relationships among apricot *S-RNase* alleles with apple (*Malus domestica*). *S-RNase* alleles (GenBank accessions: AWL24801.1, *Md-*S_{24}; AWL24810.1, *Md-*S_{58}) used as outgroup. Yellow asterisk: the groups of *S-RNase* have a close relation.

3. Discussion

The spatial distribution of *S-RNase* alleles exhibits distinct biogeographical clustering, serving as a molecular signature for tracing germplasm evolution. Our analyses revealed pronounced regional specificity. S_{66} and S_{49} mainly appear in the Xinjiang apricot population. S_{16} is present with high frequency in the north regions, including Northwest China (Figure 4 and Table S2). These differences may reflect varying environmental selection pressures or the effects of genetic drift across regions. Data from the literature indicated that the S_7-allele is only present in Southern Europe and North Africa [30]. The alleles S_{10}–S_{14} showed an Armenian origin and have also been detected in Turkish and Moroccan apricots, but are absent in Western and Southern European countries [23]. The self-compatible type, S_C, was not detected in Chinese apricots, only existing in European apricot cultivars. It is generally accepted that the genetic diversity of apricots decreases from east to southwest, and in this context, it is questionable whether S_C might be one of the causes [30]. In addition, in this study, we also found that S_8, S_9, and S_{11} appear at relatively high frequencies in both Chinese native cultivars and some European cultivars (Figure 3), which might be ancient genes of apricot cultivars.

In plum, the *S*-locus genotype is suitable for diversity studies in polyploid *Prunus* species [12]. Alburquerque et al. [31] declared that the number of *S-alleles* in apricot should be low, as only eight alleles were detected in Mediterranean and North American accessions. Halász et al. [10] identified more (at least nine) new alleles in the tested Eastern European and Central Asian genotypes, and further explained that the Central Asian eco-geographical group has a more variable genetic background compared to the European group. In this study, 43 *S-alleles* were detected among 133 Chinese apricot cultivars, and the diversity of *S-alleles* is related to the rich genetic diversity of Chinese apricot resources.

The *S*-genotype may be highly associated with certain trait characteristics of the cultivar. In this study, we found that S_{53} appears at a high frequency in white-fleshed cultivars (Table S1). Cultivars with the S_8 genotype have a higher yield, similar to that of S_C cultivars. The self-compatibility of S_8 cultivars needs further verification (Table S3). S_{66} mainly appears in the Xinjiang apricot population. A defining morphological feature of these apricot cultivars is their glabrous exocarp (fruit epidermis), characterized by a smooth cuticular structure and distinct glossiness (Table S4). For botanical classification, they are exclusively classified as *Prunus armeniaca* var. glabra Sun S.X. Supporting this point, Wu et al. [26] reported that the more frequent occurrence of these three alleles may be due to their linkage to beneficial traits or conferring adaptation to local environmental conditions. Also, in sweet cherry, certain *S-alleles* have a higher selective advantage and confer beneficial economic characteristics [32].

The *S-allele* type has been used as a means of cultivar identification. Among these tested cultivars, 'Jingzaohong' (S_9S_{36}) was a new accession developed by cross-breeding in recent years. Its female parent and pollen parent were 'Dapiantou' ($S_{36}S_{102}$ and 'Honghebao' (S_9S_{26}), respectively. 'S_9' and 'S_{36}' were inherited from 'Honghebao' (S_9S_{16}) and 'Dapiantou' ($S_{36}S_{102}$), respectively (Table 3). The present data shows good correspondence between the *S-alleles* of the parents and those inherited by the individual cultivars. Another new apricot cultivar 'Jingluofeng ($S_{11}S_{102}$)' was selected from the seeding of the cultivar 'Luotuohuang (S_8S_{11})', and 'S_{11}' was inherited from its female parent 'Luotuohuang' (S_8S_{11}). These results are from the previous report by Zhang et al. on Chinese apricots [25]. For the cultivars 'Hongfeng' (S_9S_{10}) and 'Xinshiji' (S_9S_{10}), 'S_9' and 'S_{10}' were inherited from their parents 'Honghebao' (S_9S_{11}) and 'Erhuacao' ($S_{10}S_{11}$), respectively. Common alleles may indicate a common origin [10], a notion supported by SSR markers [33].

In order to verify the homonymy in *S-RNase* naming among apricot cultivars, we analyzed the *S-alleles* identified in this study with all known synonyms from previous studies (Table S5). There are 17 cultivars in this study that were assigned *S*-genotypes in previous studies. Some cultivars, such as 'Jiguang', 'Zhanggongyuan', and 'Bergeron', had the same *S*-genotypes in this study as in previous studies, suggesting that the cultivar names are accurate. 'Qiaoerpang', 'Yinxiangbai', 'Honghebao', 'Canino', and 'Ninfa' had only one similar *S-allele*. The other nine cultivars presented completely different *S*-genotypes. These cultivars may be regarded as instances of homonymy.

China is recognized as the center of apricot origin and has an extremely abundant apricot germplasm [34]; however, few *S*-genotypes of this germplasm have been determined. In this study, *S*-genotypes of as many as 133 apricots native to China were identified. However, there were 46 cultivars that exhibited only one *S-allele* (Table 3). In this experiment, all 46 varieties were analyzed using five pairs of primers, and each primer pair consistently yielded only a single allele. A similar result was reported by Boubakri et al. in a study on Tunisian apricot cultivars, in which only one *S-allele* was detected [23]. However, the underlying reasons for this observation remain unclear. One possibility is that large intron fragments within the *S-RNase* of these cultivars may hinder effective amplification [26], making the current primers less suitable for these specific genotypes. Additionally, some cultivars have complex genetic backgrounds. For instance, 'Jingren No.2', 'Jingren No.1', 'Jingren No.3', 'Jingren No.4', and 'Jingren No.5' were all derived from distant hybridization between apricot (*Prunus armeniaca*) and almond (*Prunus amygdalus*) [35]. Although the primers used are generally applicable across *Prunus* species, designing primers based on specific sequence features and screening optimal primer combinations may improve amplification efficiency in these genetically complex accessions. Another plausible explanation is that some cultivars exhibit homozygosity at the S-locus, a phenomenon previously reported

in *Prunus* species [10]. To accurately determine the *S*-haplotypes of these undetected alleles, more advanced genomic approaches such as long-read sequencing should be employed.

Currently, comprehensive *S*-genotype data can provide scientific guidance for apricot production. First, *S*-genotype data enables the creation of empirically validated cross-incompatibility matrices for optimized orchard pollinizer design. These cross-compatibility matrices, which group cultivars by *S*-genotype, directly support pollinizer selection (Table S6). Secondly, this data provides a molecular foundation for strategic parental selection in apricot breeding programs, particularly for developing self-compatible cultivars through specific *S-alleles*. The newly identified *S-RNase* genes substantially expand the known allele diversity within *P. armeniaca* and provide new molecular markers for phylogenetic studies of *S*-locus evolution in *Rosaceae*.

4. Materials and Methods

4.1. Plant Materials

A total of 168 apricot accessions with known and unknown compatibility phenotypes were analyzed in this study. The collection comprised 133 Chinese cultivars and 35 international accessions, including 32 European cultivars, 1 American accession, and 2 Japanese genotypes. These cultivars were obtained from the apricot germplasm collection of the Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences.

4.2. DNA Extraction

Total genomic DNA was extracted from young leaves using the Hi-DNAsecure Plant kit DP350-03 (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The concentration of the isolated DNA was determined using a Thermo Scientific NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA.) and via electrophoresis on 1% agarose gels.

4.3. PCR Amplification

Five primer pairs, previously reported as universal primer combinations for *Prunus* plants, were used to perform the specific PCR amplification of *S-alleles*: EM-PC2consFD+EM-PC3consRD, PruC2+Amy-C5R, PruC2+PCE-R, As1II+AmyC5R, and PaConsII-F+PaConsII-R. The specific primer sequences are shown in Table 4. PCR cycling parameters and conditions were as described in the respective references.

Table 4. Sequences of five primer pairs used for *S-RNase* gene amplification.

Number	Primer Name	Sequence (5′ to 3′)	References
1	EM-PC2consFD EM-PC3consRD	TCACM * ATYCATGGCCTATGG AW * CTR * CCRTGY * TTGTTCCATTC	Sutherland et al., 2004 [36]
2	Pru-C2 Pru-C5	CTATGGCCAAGTAATTATTCAAACC TACCACTTCATGTAACAACTGAG	Tao et al., 1999 [37]
3	Pru-C2 PCE-R	CTATGGCCAAGTAATTATTCAAACC TGTTTGTTCCATTCGCCTTCCC	Tao et al., 1999; Wu et al., 2009 [26,37]
4	AS1II AmyC5R	TATTTTCAATTTGTGCAATGG CAAAATACCACTTCATGTAACAAC	Tamura et al., 2000 [16]
5	PaCons II-F PaCons II-R	GGCCAAGTAATTATTCAAACC CATAACAAARTACCACTTCATGTAAC	Sonneveld et al., 2003 [14]

* M = A/C; Y = C/T; W = A/T; and R = A/G.

4.4. Cloning and Sequencing of S-Alleles

The PCR-amplified fragments were excised from 1.2% agarose gels and purified using the Agarose Gel DNA Purification Kit (TaKaRa, Dalian, China). The purified products were cloned into the pEASY-Blunt Simple Cloning vector (Tiangen Biotech, Beijing, China) following the manufacturer's instructions and transformed into *Escherichia coli* DH5α. To obtain an accurate sequence and avoid errors caused by PCR, three independent positive clones of each fragment were sequenced by Sangon Biotech Company (Shanghai, China).

To identify the S_C-haplotype, a two-step approach was used, as described by Halász et al. [5]. For the first step, an allele-specific reverse primer, AprSC8-R, was used in combination with PaConsI-F [14] to amplify the S_C/S_8-RNase allele. For the second step, specific primers, AprFBC8-F and AprFBC8-R, were designed selectively to amplify the SFBC/8 alleles [38].

4.5. Analysis for Sequence Data and Identification of S-Alleles

DNAMAN 8 software was employed for multiple sequence alignment and annotation of putative *S-alleles*. Nucleotide sequences were subjected to homology analysis using BLASTN against the NCBI nucleotide database. Intron–exon boundaries were determined through comparative alignment of genomic DNA with corresponding DNA references from *Prunus armeniaca S*-alleles. The translated amino acid sequences spanning the conserved C2–C5 domains were derived from the annotated nucleotide data. Subsequent protein-level verification employed BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 20 February 2025)) to compare deduced amino acid sequences against the NCBI non-redundant database.

4.6. Construction of Phylogenetic Tree Based on S-RNase Gene Sequences

All the predicted amino acid sequences between the C2 and C5 regions from 46 detected *S-RNase* genes were aligned with each other using the ClustalW algorithm in MEGA 11, and a neighbor-joining tree was constructed. Two apple (*Malus domestica*) *S-RNase* alleles (*Md-*S_{24}: AWL24801.1; *Md-*S_{58}: AWL24810.1) were used as outgroup. The Poisson correction method was used to compute evolutionary distances, and the reliability test was performed 1000 times using Bootstrap.

5. Conclusions

In this study, the *S*-genotypes of 168 apricot cultivars were determined via cloning and sequencing the specific PCR products. A total of 46 different *S-RNase* alleles, with 31 previously reported and 15 new alleles, were identified. The self-compatible type, S_C, was not detected in the 133 Chinese accessions tested. Then, the *S-allele* frequency distribution patterns were investigated, and the results indicated that S_8 emerged as the predominant *S-allele* across all tested apricot accessions, followed sequentially by S_{11}, S_9, S_{16}, and S_{53}. The geographic distribution patterns of *S-allele* frequencies in Chinese apricot cultivars were also analyzed. The most frequent alleles in Northern China are S_8, S_{11}, and S_{53}. In the northwestern region of China, S_{16} was the dominant *S-RNase* gene. Based on the *S-RNase* gene sequence data, the phylogenetic tree of apricot *S-alleles* was constructed. These results can benefit future breeding programs by aiding the selection of appropriate parents and can contribute to efficient orchard design by promoting the planting of cross-compatible apricot cultivars.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms26178667/s1.

Author Contributions: J.Z. conceived of the study, performed most of the experiments, analyzed the data, and wrote the manuscript. M.Z. participated in S_C detection. W.Y. and F.J. participated in graphical refinement. L.Y., J.Z. and J.L. collected plant material. H.S. conceived of the study and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the National Key Research and Development Program of China (No. 2023YFD2200305).

Data Availability Statement: The data is contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no competing interests.

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