

報 文

Upgrading the search system for apple cultivar combinations for breeding and cultivation: *S*-RNase allele addition/modification and multilingual localization

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Abstract

We searched the NCBI and DDBJ database to collect *S*-RNase alleles in apple, and then confirmed and unified their numbering. We identified an *S*-RNase allele genotype of ‘Shandingzi’ as *S*₃₃*S*₄₁ from the phylogenetic analyses of *S*-RNase alleles. We corrected *S*_{6a} to *S*₆, and *S*_{6b} to *S*₁₇, and then added *S*₃₉, *S*₄₁, *S*₄₅, *S*₄₆, *S*₅₀, *S*₅₈, and *S*₅₉ as new *S*-RNase alleles on our database of *S*-RNase alleles. We upgraded and/or constructed the English, Japanese, Chinese, Italian, Portuguese, Russian, and Belarusian versions of a searching system for apple cultivar combinations showing incompatible, semi-compatible, and compatible.

Keywords : Apple, Self-incompatibility, *S*-RNase allele

1. Introduction

As apple (*Malus x domestica* Borkh.) has a gametophytic self-incompatibility (GSI) system, two genetically distinct cultivars are necessary for fruit production. Apple GSI is controlled by the multi-allelic *S*-locus, and pollen tube growth is inhibited in cases where the *S*-haplotype of the pollen matches either of the *S*-haplotypes from the diploid pistil (Kobel et al. 1939; de Nettancourt 1977). In the apple GSI, two tightly-linked polymorphic genes, pollen *S*; *SLF* (*S*-

locus F-box)/*SFB* (*S*-haplotype-specific F-box) and pistil *S*; *S*-RNase (*S*-Ribonuclease), act as pollen and pistil factors, respectively (Broothaert et al. 1995; de Franceschi et al. 2012). Since two cultivars with no common *S*-haplotype are necessary for the apple fertilization, accurate knowledge of the *S*-genotype is important. We developed a search system for suitable apple combinations for cultivation and breeding based on *S* genotypes using the sequence information of the *S*-RNase allele involved in apple self- and cross-

compatibility (<http://www.agr.nagoya-u.ac.jp/~hort/apple/>) (Matsumoto 2014). Furthermore, we developed a search-system to find combinations of apple cultivars for efficient breeding of red-fleshed apples by using the linkage between red-fleshed apple traits and *S*-genes (<http://www.agr.nagoya-u.ac.jp/~hort/apple/red/>)

(Matsumoto et al. 2018). In this report, we describe the enhancement of the *S* genotype database and the upgrade and/or construction of the search system in English, Japanese, Chinese, Italian, Portuguese, Russian, and Belarusian.

2. Materials and Methods

A total of 305 *S-RNase* alleles were obtained from the apple whole genome information and *S-RNase* registry information. The *S* allele designations were unified through phylogenetic analysis (Fig. 1). To build a phylogenetic tree, protein sequences were aligned by using ClustalW (ver. 2.1; Larkin et al., 2007) with default parameters, followed by estimation of the tree using MEGA (ver.10.1.8; Kumar et al., 2018) with 1000 times bootstrap testing. The *S* genotype information for a total of 1168 cultivars, which were added or modified based on literature information, in addition to 150 Chinese apple cultivars whose *S* genotypes were newly identified, were converted to electronic files. Lastly, a search engine using JavaScript was constructed to facilitate the search of *S* genotype information.

3. Results and Discussion

3-1. Construction of an *S-RNase* allele database

Apple self-incompatibility is controlled by a single locus, called the *S*-locus, consisting of two closely linked alleles, *S-RNase* and *SFB*. *S-RNase* acts as a pistil factor and single gene; but, *SFB* acts as a pollen factor with multiple genes. We have corrected deduced protein sequences of *S-RNase* genes by searching the database. In the case of complete sequences, we used

their allele numbers for *S*-haplotype numbers, with some rearrangement.

Based on the phylogenetic analysis of the *S-RNase* allele of the apple cultivar ‘Shandingzi’ for which whole genome information has been released, its *S* genotype was assigned as *S*₃₃*S*₄₁ (Fig. 2).

In addition, since it was shown that *S*_{6a} and *S*_{6b}, which had been considered to be the same allele, may actually be different alleles, *S*_{6a} was revised to *S*₆ and *S*_{6b} to *S*₁₇, and *S*₃₉, *S*₄₁, *S*₄₅, *S*₄₆, *S*₅₀, *S*₅₈, and *S*₅₉ were added as novel *S-RNase* alleles (De Franceschi et al., 2019) (Table 1).

Table 1 *S*-haplotypes and *S-RNase* alleles of apple cultivars.

Proposed <i>S</i> -haplotype	<i>S-RNase</i> allele	Cultivar	<i>S-RNase</i> genotype
6	6a	‘Mela Rozza’	<i>S</i> ₃ <i>S</i> ₆
17	6b	‘Heta’	<i>S</i> _{16b} <i>S</i> ₁₇
		‘Maikki’	<i>S</i> ₁₀ <i>S</i> ₁₇
39	39	‘Young America’	<i>S</i> ₂₈ <i>S</i> ₃₉
41	41	‘Murray’	<i>S</i> ₁₀ <i>S</i> ₄₁
45	45b	‘Adirondack’	<i>S</i> ₃₀ <i>S</i> _{45b} <i>S</i> ₄₆
		‘Snowdrift’	<i>S</i> ₂₅ <i>S</i> _{45b}
46	46	‘Ijunscoe Ranee’	<i>S</i> ₃₉ <i>S</i> ₄₆
50	50	‘Durello di Forli’	<i>S</i> ₃ <i>S</i> ₅₀
58	58	‘Åkerö’	<i>S</i> ₁ <i>S</i> ₅₈
		‘Antonovka Pamtorutka’	<i>S</i> ₈ <i>S</i> ₅₈
59	59	‘Doubloons’	<i>S</i> ₄ <i>S</i> ₅₉

S genotypes of ‘Åkerö’, ‘Braeburn’, ‘Burgundy’, ‘Delicious’, ‘Filippa’, ‘Fuji’, ‘Jonathan’, ‘Lady Williams’, ‘Macoun’, ‘Mcintosh’ (which was determined to be the same variety as ‘McIntosh’), ‘Spässerud’, ‘Stark Earliblaze’, ‘Winesap’, and ‘York Imperial’ were consistent with those already reported and were deleted from the database to avoid duplication (De Franceschi et al., 2019; Kim et al., 2008). ‘Dr. Oldenburg’ was determined to be a variety different from ‘Oldenburg’ and was left in the database (De Franceschi et al. 2019). ‘Priscilla’ and ‘Worcester Pearmain’ were left in the database as ‘Priscilla_Italy’ (*S*₇*S*₁₀) and ‘Worcester Pearmain_Italy’ (*S*₂₄*S*₂₅) because they have a different *S* genotype from ‘Priscilla’

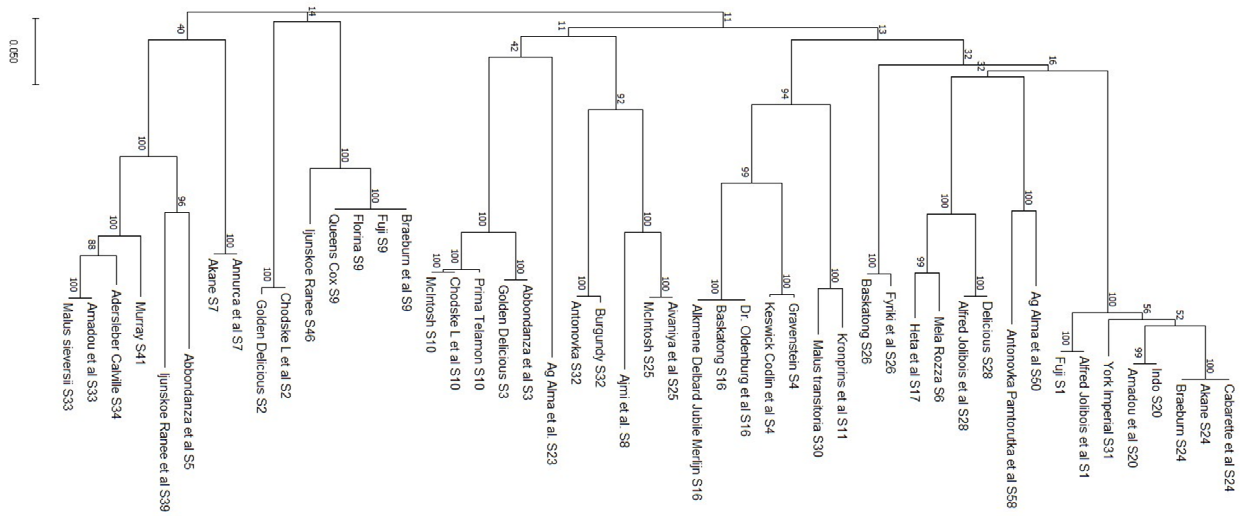


Figure 1. Unrooted Neighbor-Joining tree of the previously reported *S-RNase*. Local bootstrap probabilities are shown next to the branches. Scale bar corresponds to 0.05 amino acid substitution per residue.

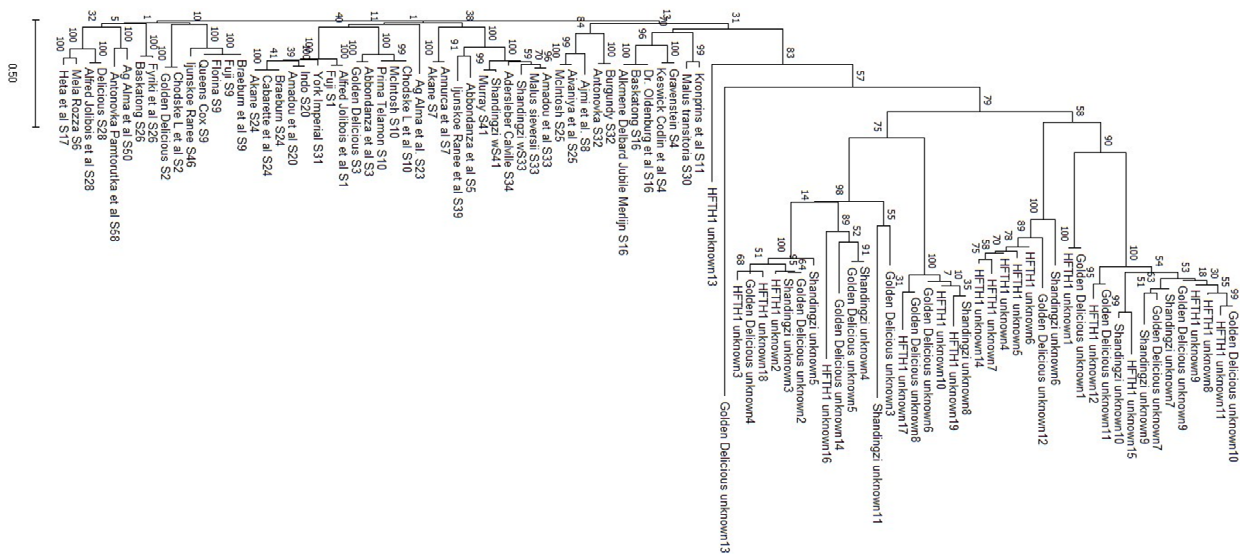


Figure 2 Unrooted Neighbor-Joining tree of *S-RNase* and *S-RNase*-like proteins collected in this report. Local bootstrap probabilities are shown next to the branches. Scale bar corresponds to 0.5 amino acid substitution per residue.

(S_9S_{20}) and ‘Worcester Pearmain’ (S_2S_{24}) (De Franceschi et al., 2019). ‘Benoni’ also was left in the database as ‘Benoni_India’ (S_1S_{24}) since it differs from ‘Benoni’ (S_5S_{11}) in *S* genotype (Mir et al., 2016). *S*-genotypes of ‘Charden’ (S_3S_x), ‘Indian Summer’ ($S_{26}S_x$), and ‘Snowdrift’ ($S_{25}S_x$) were corrected to ‘Charden’ ($S_2S_3S_4$), ‘Indian Summer’ ($S_{26}S_{50b}$), and ‘Snowdrift’ ($S_{25}S_{45b}$) (Kim et al., 2008; Sheick et al., 2018).

3-2. Search system for suitable apple cultivar combinations

We have updated the database of *S-RNase* for English, Japanese, Chinese, Italian, and Portuguese versions of the search system. We have newly added Russian and Belarusian versions of the search system

(Fig. 3). Users can access those homepages at the sites listed in the next paragraph. To find and select a target cultivar, users can write the first letter of a cultivar name in the search box, and a list of similar cultivar names will appear automatically. After selecting the target cultivar, users must decide whether to search for an ovule parent or a pollen parent, and then click the search button. The search system indicates the apple cultivars showing full incompatibility, semi-compatibility, and full compatibility against the target cultivar (Fig. 3). Once used, the clear button must be pressed before the next use.

The English version of the search system is available at <http://www.agr.nagoya-u.ac.jp/~hort/apple/>, the Japanese version at <http://www.agr.nagoya-u.ac.jp/~hort/apple/ja/>, the Chinese version at <http://www.agr.nagoya-u.ac.jp/~hort/apple/ch/>, the Italian version at <http://www.agr.nagoya-u.ac.jp/~hort/apple/it/>, the Portuguese version at <http://www.agr.nagoya-u.ac.jp/~hort/apple/pt/>, the Russian version at <http://www.agr.nagoya-u.ac.jp/~hort/apple/ru/>, the Belarusian version at <http://www.agr.nagoya-u.ac.jp/~hort/apple/by/>.

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References

- Broothaert M, Janssens GA, Proost P, Broekaert WF (1995) cDNA cloning and molecular analysis to two self-incompatibility alleles from apple. *Plant Mol Biol* 27:499-511.
- De Franceschi P, Dondini L, Sanzole J (2012) Molecular bases and evolutionary dynamics of self-incompatibility in the Pyrinae (Rosaceae). *J Exp Bot* 63:4015-4032.
- De Franceschi P, Bianco L, Cestaro A, Dondini L, Velasco R (2018) Characterization of 25 full-length *S-RNase* alleles, including flanking regions, from a pool of resequenced apple cultivars. *Plant Mol Biol* 97:279-296.
- De Franceschi P, Bianco L, Cestaro A, Dondini L, Velasco R (2019) Data mining for apple *S-RNase* alleles in resequencing datasets. *Acta Hort* 1231:135-142.
- De Nettancourt D (1977) Incompatibility in angiosperms, in: Frankel R, Gal GAE, Linskens HF (Eds.) *Monographs on Theoretical and Applied Genetics*. Springer, Heidelberg, Germany, pp. 28-57.
- Kim H, Park J, Hirata Y, Nou I (2008) Molecular characterization of new *S-RNases* ('*S₃₁*' and '*S₃₂*') in apple (*Malus X domestica* Borkh.). *J Plant Biol* 51:202-208.
- Kobel F, Steinegger P, Anliker J (1939) Weitere Untersuchungen über die Befruchtungsverhältnisse der Apfel- und Birnsorten. *Land-wirtsch Jahrb Schweiz* 53:160-191.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol* 35:1547-1549.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F,

- Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947-2948.
- Matsumoto S, Tianzhong L, Otagaki S, Yang L, Songling B (2018) Efficient breeding and cultivation of type 2 red-fleshed apple cultivars using a search system for suitable apple cultivar combination. *Hortic Plant J* 4:219-225.
- Matsumoto S (2014) Apple pollination biology for stable and novel fruit production: search system for apple cultivar combination showing incompatibility, semicompatibility, and full-compatibility based on the *S-RNase* allele database. *Int J Agron Article ID* 138271.
- Mir JI, Ahmed N, Singh DB, Sheemar G, Hamid A, Zaffer S, Shafi W (2016) Molecular identification of *S*-alleles associated with self-incompatibility in apple (*Malus* spp.) genotypes. *Indian J Agric Sci* 86:78-81.
- Sheick R, Serra S, De Franceschi P, Dondini L, Musacchi S (2018), Characterization of a novel self-incompatibility allele in *Malus* and *S*-genotyping of select crabapple cultivars. *Sci Hortic* 240:186-195.

育種・栽培に資するリンゴ交配品種組み合わせ検索システムの バージョンアップ: *S* 遺伝子型の追加・修正と多言語対応.

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要 旨

リンゴの *S-RNase* 対立遺伝子情報について, NCBI と DDBJ のデータベースを検索し, 遺伝子番号を確認するとともに統一した. *S-RNase* 対立遺伝子の系統解析から, 'Shandingzi' の *S* 遺伝子型を $S_{33}S_{41}$ と同定した. S_{6a} を S_6 に, S_{6b} を S_{17} に修正し, S_{39} , S_{41} , S_{45} , S_{46} , S_{50} , S_{58} , S_{59} を新たな *S-RNase* allele として *S-RNase* allele データベースに追加した. また, 英語, 日本語, 中国語, イタリア語, ポルトガル語, ロシア語, ベラルーシ語版のリンゴ品種組み合わせ検索システム(不和合, 半和合, 和合)をアップデートもしくは新たに構築した.