Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2016, Article ID 3101864, 5 pages
http://dx.doi.org/10.1155/2016/3101864

Research Article

Apple Cultivation and Breeding in Afghanistan: *S-RNase* Genotypes and Search System for Suitable Cultivar Combination

Matiullah Akbari,[1] Mao Yamaguchi,[1] Tsutomu Maejima,[2] Shungo Otagaki,[1] Katsuhiro Shiratake,[1] and Shogo Matsumoto[1]

[1]*Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan*
[2]*Nagano Fruit Tree Experiment Station, Nagano 382-0072, Japan*

Correspondence should be addressed to Shogo Matsumoto; shogo@agr.nagoya-u.ac.jp

Received 21 October 2015; Accepted 30 December 2015

Academic Editor: Othmane Merah

We investigated *S-RNase* genotypes of nine useful Afghan apple cultivars including six original cultivars and one rootstock. We also determined *S-RNase* genotypes of 11 apple cultivars and lineages and seven rootstocks in Japan. We speculated regarding the unidentified parents of cultivars and lineages from the *S-RNase* genotypes and their fruit and branch characteristics and also identified mistaken parents. We compiled a database of the apple *S-RNase* genotypes of 622 apple cultivars investigated, which included a survey system of cultivar combinations showing those that were fully incompatible, semicompatible, and fully compatible, written in the Pashto language.

1. Introduction

The origin of apple is in Central Asia, and many areas of Afghanistan have native apples. Apple is one of the most cultivated horticultural crops in this country since the climate is appropriate for it. The major areas of apple production, that is, areas producing more than 1,000 metric tons per year, are Ghazni, Kabul, Kandahar, Kapisa, Logar, Paktika, Paktya, Panjshir, Parwan, Uruzgan, and Wardak [1]. According to the forecast of the Ministry of Agriculture, Irrigation, and Livestock (MAIL), apple production in Afghanistan increased five to 10% from 2012 to 2013 [1]. It was 77,000 metric tons in 2013/14 (April/March) [1].

Self-incompatibility is a genetic mechanism that prevents inbreeding and promotes outcrossing in many flowering plants [2]. The gametophytic self-incompatibility (GSI) system in apple is controlled by the multiallelic *S*-locus [2, 3], so that when the pollen *S*-haplotype matches one of the pistil *S*-haplotypes, the pollen tube growth is arrested in the style. In apple GSI, F-box gene called SFBB (S-locus F-box brothers) and *S-RNase* gene act as pollen and pistil factors, respectively [4, 5]. As fertilization of apple needs at least two cultivars having no or one common *S*-haplotype, accurate knowledge of the *S*-genotype that is identified by the *S-RNase* alleles is important for stable apple production.

Afghan apple growers know about the self-incompatibility of apple cultivars, but unfortunately they know nothing about their *S-RNase* allele genotypes. Currently in Afghanistan, the apple growers design their apple orchard based on the fruit skin colors of different apple cultivars. However, sometimes the combination of two apple cultivars having different skin color results in full-incompat-ibility due to their identical *S*-genotype, like S_2S_3 of "Jester" (red skin) and "Golden Delicious" (yellow skin). Current apple production in Afghanistan is mainly from exotic cultivars imported around 20 years ago [1]. Among various apple cultivars including "Discovery" ($S_{10}S_{24}$) [6], "Fiesta" (S_3S_5) [7], "Fuji" (S_1S_9) [8, 9], "Gala" (S_2S_5) [7], "Golden Delicious" (S_2S_3) [7], "Granny Smith" (S_3S_{23}) [6], "Jester" (S_2S_3) [10], "July Red" ($S_{24}S_{25}$) [http://www.agr.nagoya-u.ac.jp/~hort/apple/], "Mutsu" ($S_2S_3S_{20}$) [9, 11], "Double Red Delicious" (S_9S_{28}) [12], "Red Devil," "Saturn," "Spartan" (S_9S_{10}) [13], and "Summerred" (S_2S_9 in Belgium, $S_{10}S_{24}$ in Japan) [6, 14], three cultivars "Double Red Delicious," "Golden Delicious," and "Granny Smith" (known as Green Delicious in Afghanistan) are mainly grown commercially [15]. Though the cost of

(a) (b) (c) (d)

Figure 1: A mature fruit and a shape of tree of "Natsuotome" ((a), (b)) and "Swakko" ((c), (d)) produced at Nagano Fruit Tree Experiment Station.

apple production in Afghanistan is lower than in other countries, their apple quality is higher than the ones imported from Pakistan or Iran. Developing original new and high-quality cultivars using Afghan original cultivars seems to be important for Afghan apple growers who wish to compete in local and international markets.

In this study, we investigated the *S*-genotypes of apple cultivars and lineages including Afghan original cultivars for apple breeding and cultivation. In addition, we translated the apple *S-RNase* allele genotype database to the Pashto language and made a homepage where Afghan apple growers can search for suitable cultivar combination. Indeed, Pashto is Afghanistan's official language and is used by more than 60% of the country's populations.

2. Materials and Methods

2.1. Plant Material. Young leaves of six Afghan original cultivars and a rootstock, that is, "Jawrasi Golden," "Kado Seb," "Mahali" (rootstock), "Nazak Badan," "Parachinari," "Swati," "Tur Kulu," "Red Devil," and "Saturn" [15, 16], were collected from either Badam Bagh, Kabul Province, or Wardak Province in Afghanistan. Remaining apple cultivars, lineages, and rootstocks used in this study were from collections at the Iwate Agricultural Research Center, Japan, the Nagano Fruit Tree Experiment Station, Japan, or Koukaen, Esashi-ku, Oshu City, Japan. Young leaves were stored at −80°C until use.

2.2. S-RNase Allele Amplification and Allele-Specific PCR-RFLP Analyses. Total DNA was isolated from the leaves of individual plants [17]. The primers and conditions used for *S*-allele-specific PCR-RFLP analyses were those described by Broothaerts et al. (for S_2 and S_3) [18], Janssens et al. (for S_7 and S_9) [7], Kitahara et al. (for S_{24}) [19], Kitahara and Matsumoto (for S_{10}) [9], Matsumoto et al. (for S_5 and S_7) [20], Matsumoto et al. (for S_1 and S_{20}) [21], and Matsumoto and Kitahara (for S_{28}) [22].

3. Results and Discussion

3.1. S-RNase Genotypes of Apple Cultivars Including Afghanistan Original Cultivars. We analyzed the *S-RNase* genotypes of apple cultivars and lineages for using them in a breeding programme. *S-RNase* genotypes of "Iwate 4 Gou," "Natsuakari," K-20, K-21, K-22, KAD-14, KAD-17, KAD-19, KAD-20, and "Saturn" (Table 1, numbers 1, 2, 6–8, 12–15, and 25) matched one of the *S-RNase* genotypes deduced from their reputed parentage. In contrast, *S-RNase* genotypes of "Oirase," K-23, K-24, K-25, and "Red Devil" (Table 1, numbers 4, 9–11, and 24) were inconsistent with the one from its reputed parents. "Natsuotome" (Table 1, number 3) was identified as S_7S_9, and it was suggested that "Fuji" (S_1S_9) or "Alps Otome" (S_1S_9) seemed to be used as a paternal parent, judging from its fruit and branch characteristics. As the average fruit weight of "Natsuotome" (195 g) is lighter than that of its maternal parent "Sansa" (280 g), its characteristics might be from "Alps Otome" (50 g), not from "Fuji" (300 g). On the other hand, the characteristics of concentrically cracking at peduncle end in "Natsuotome" are similar to "Shinano Hoppe" (Akane × Fuji), a pedigree of "Fuji" (Figure 1). "Oirase" (S_9S_{28}) and K-24 (S_1S_3) (Table 1,

Table 1: *S-RNase* genotypes of 20 apple cultivars and 7 rootstocks including their lineage.

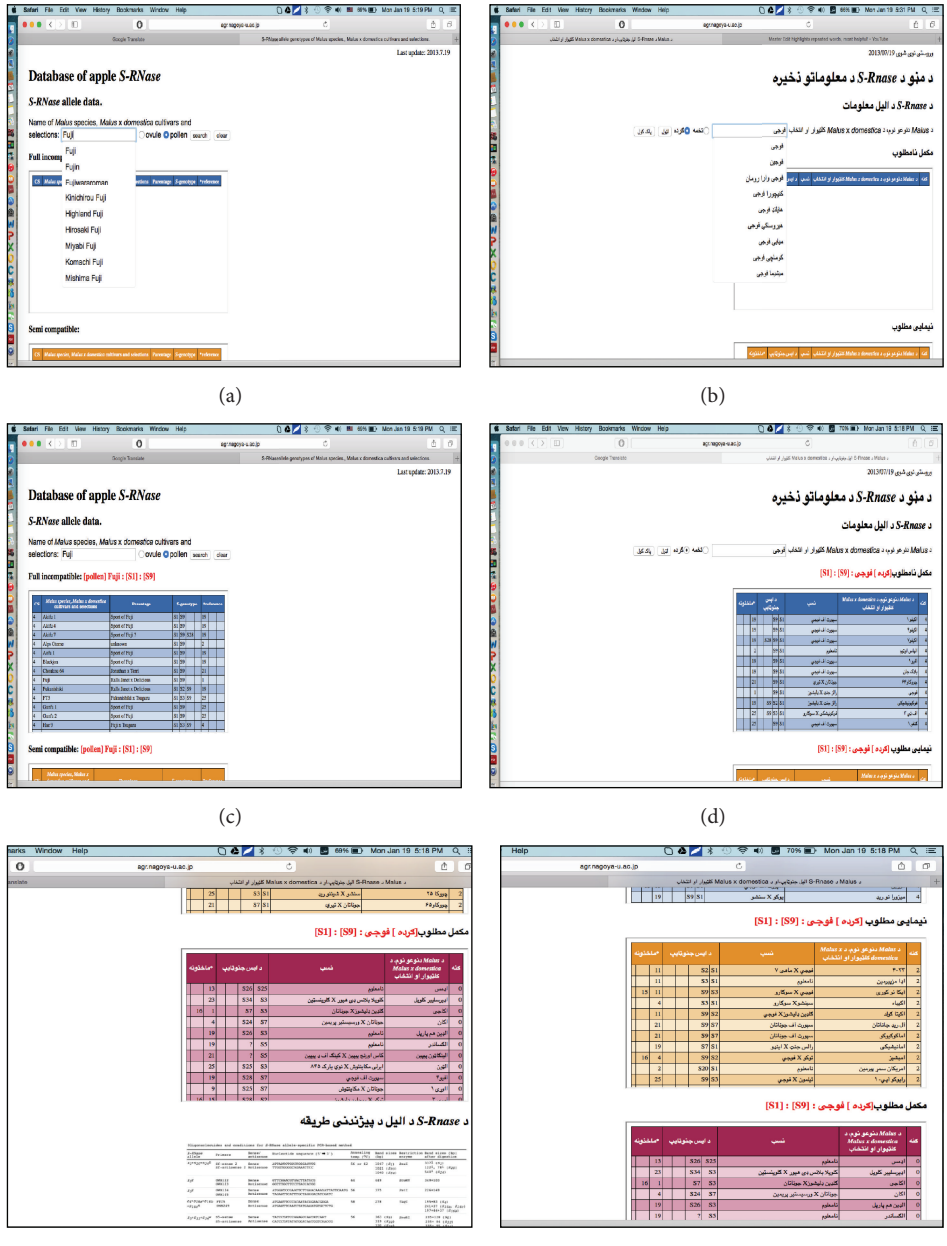
Number	Cultivar	Reputed parentage	*S-RNase* genotype
1	"Iwate 4 Gou"	"Fuji" (S_1S_9) × "Delicious" (S_9S_{28})	S_1S_{28}
2	"Natsuakari"	"Sansa" (S_5S_7) × "Yoko" (S_3S_9)	S_3S_7
3	"Natsuotome"	Chance seedling of "Sansa" (S_5S_7)	S_7S_9
4	"Oirase"	"Starking Delicious" (S_9S_{28}) × "**Tsugaru**"$^{+}$ (S_3S_7)	S_9S_{28}
5	"Suwakko"	"Sekaiichi" (S_3S_9) × Unknown$^{\neq}$	S_3S_{20}
6	K-20	"Shinano Gold" (S_1S_3) × "Beniroman" (S_3S_5)	S_1S_5
7	K-21	"Shinano Gold" (S_1S_3) × "Beniroman" (S_3S_5)	S_1S_5
8	K-22	"Orin" (S_2S_7) × "Beniroman" (S_3S_5)	S_3S_7
9	K-23	"**Gunma Meigetsu**"$^{+}$ (S_1S_3) × "**Shinano Gold**"$^{+}$ (S_1S_3)	S_1S_x
10	K-24	"Shinano Gold" (S_1S_3) × **Unknown**$^{+\neq}$	S_1S_3
11	K-25	"**Shinano Gold**"$^{+}$ (S_1S_3) × "Fuji" (S_1S_9)	S_9S_x§
12	KAD-14	JM1 (S_9S_x) × Mark (S_3S_x)	S_3S_9
13	KAD-17	JM1 (S_9S_x) × Mark (S_3S_x)	S_3S_9
14	KAD-19	JM1 (S_9S_x) × Mark (S_3S_x)	S_3S_9
15	KAD-20	JM7 (S_9S_x) × Mark (S_3S_x)	S_3S_9
16	JM1	*Malus prunifolia* × M.9 (S_3S_9)	S_9S_x§
17	JM7	*Malus prunifolia* × M.9 (S_3S_9)	S_9S_x§
18	Mark	M.9 (S_3S_9) × Unknown$^{\neq}$	S_3S_x§
19	"Jawrasi Golden"	Unknown¶ × Unknown$^{\neq}$	$S_{28}S_x$§
20	"Kado Seb"	Unknown¶ × Unknown$^{\neq}$	$S_{28}S_x$§
21	"Mahali"	Unknown¶ × Unknown$^{\neq}$	S_5S_{28}
22	"Nazak Badan"	Unknown¶ × Unknown$^{\neq}$	S_2S_9
23	"Parachinari"	Unknown¶ × Unknown$^{\neq}$	S_xS_y§
24	"Red Devil"	"Discovery" ($S_{10}S_{24}$) × "**Kent**"$^{+}$ (S_3S_9)	S_2S_{24}
25	"Saturn"	"Jonathan" (S_7S_9) × "Golden Delicious" (S_2S_3)	S_2S_7
26	"Swati"	Unknown¶ × Unknown$^{\neq}$	S_3S_{28}
27	"Tur Kulu"	Unknown¶ × Unknown$^{\neq}$	S_2S_{28}

$^{+}$Incorrect parentage indicated by this work is in bold.
$^{\neq}$Unknown: pollen parent is unknown.
$^{\S}S_x, S_y \neq S_1, S_2, S_3, S_5, S_7, S_9, S_{10}, S_{20}, S_{24}, S_{28}$.
¶Unknown: ovule parent is unknown.

numbers 4 and 10) might be a sport of "Starking Delicious" (S_9S_{28}) and "Shinano Gold" (S_1S_3), respectively, judging from their *S*-genotypes. The reputed paternal parent "Tsugaru" for "Oirase" and an unknown pollen parent for K-24 seemed to be unrelated to their production. The paternal parents of "Suwakko" (Table 1, number 5) are unknown. "American Summer Pearmain" (S_1S_{20}) or "Indo" (S_7S_{20}) or "Kitanosachi" (S_7S_{20}) might be used as a paternal parent of "Suwakko" (S_3S_{20}), given its *S-RNase* genotype and resembling its branch characteristics to "Indo" (Figure 1). In the case of K-23 (Table 1, number 9), either its maternal or its paternal parents were wrong. Since the *S-RNase* genotype of K-25 (Table 1, number 11) was identified as S_9S_x, its maternal parent "Shinano Gold" (S_1S_3) might have been wrongly identified. One unknown *S-RNase* allele was present in three (JM1, JM7, and Mark [Table 1, numbers 16–18]) out of seven rootstocks in Japan.

Within the six original Afghan cultivars, "Nazak Badan," "Swati," and "Tur Kulu" (Table 1, numbers 22, 26, and 27) were determined as S_2S_9, S_3S_{28}, and S_2S_{28}, respectively. We also identified *S*-genotypes of rootstock "Mahali" (Table 1, number 21) as S_5S_{28}, but one or two unknown *S-RNase* alleles were present in "Jawrasi Golden" ($S_{28}S_x$), "Kado Seb" ($S_{28}S_x$), and "Parachinari" (S_xS_y) (Table 1, numbers 19, 20, and 23). This is the first case to analyze *S*-genotypes of Afghan cultivars.

3.2. Database of S-RNase Allele Genotypes and the Search System for Suitable Apple Cultivar Combination Using the Pashto Language. We previously established a search system for apple cultivar combination, written in English on the homepage (http://www.agr.nagoya-u.ac.jp/~hort/apple/) [23]. In this study, we have developed a Pashto language version of the search system homepage for apple growers in Afghanistan. At first all names of cultivars and their parents in the database were translated from English to the Pashto language. Then, we changed the search system homepage to a Pashto language version. As the writing of the Pashto language reads from right to left, the new homepage looks like an opposite of the English version (Figure 2).



(a) (b)

(c) (d)

(e) (f)

FIGURE 2: Pashto language version of representative screenshots of the search system. "Fuji" was selected for searching cultivars on the English version (a) and Pashto version (b). Search results: cultivars incompatible with "Fuji" on the English version (c) and Pashto version (d), cultivars semicompatible with "Fuji" on the Pashto version (e), and cultivars fully compatible with "Fuji" on the Pashto version (f).

All apple growers in Afghanistan can access the homepage at the URL (http://www.agr.nagoya-u.ac.jp/~hort/apple/ps/). In the homepage of this database, there is a box called the search box. When you write the first letter of a cultivar name in the search box, a list of similar cultivar names will appear from which you can select the target cultivar easily (Figures 2(a) and 2(b)). Afterward, before clicking the search button, you must decide if the cultivar is used as an ovule parent or pollen parent. Then click the search button. Actually, the search system of *S*-genotype database is developed for suitable combinations of apple cultivars. The search system of *S*-genotype database indicates three different types of combinations between apple cultivars: full-incompatibility, semicompatibility, and full-compatibility. Therefore, you will find three different open windows reflecting the 3 compatibilities mentioned above (Figures 2(c)–2(f)).

4. Conclusions

We have investigated 27 *S-RNase* genotypes of apple cultivars and lineages including the nine most useful Afghanistan apple cultivars. We developed a Pashto language version of

the *S*-genotypes database of apple cultivars, along with a convenient search system for cultivar combination showing three different types of compatibilities, that is, full-compatibility, semicompatibility, and full-incompatibility. The translated Pashto version of the search system will enable apple growers in Afghanistan to select suitable and compatible apple cultivars for successful combination in an orchard to ensure stable fruit production and breeding.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors are indebted to Mr. T. Okawa for his technical advice in developing a DNA database with a search capability and to Mr. T. Takano and Mr. T. Asakawa for supplying the samples for new apple cultivars and lineages. This research was carried out under the Project for the Promotion and Enhancement of the Afghan Capacity for Effective Development (PEACE).

References

[1] J. Safi and D. Bunnel, "Afghanistan's vibrant apple market," Global Agricultural Information Network, USDA Foreign Agricultural Service, 2013, http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Afghanistan's%20Vibrant%20Apple%20Market_Kabul_Afghanistan_11-18-2013.pdf.

[2] D. de Nettancourt, *Incompatibility in Angiosperms*, Monographs on Theoretical and Applied Genetics, Springer, Heidelberg, Germany, 1977, edited by: R. Frankel, G. A. E. Gal, H. H. Linskens.

[3] F. Kobel, P. Steinegger, and J. Anliker, "Weitere untersuchungen über die befruchtungsverhältnisse der apfel- und birnsorten," in *Landwirtschaftliches Jahrbuch der Schweiz*, vol. 53, pp. 160–191, 1939.

[4] W. Broothaerts, G. A. Janssens, P. Proost, and W. F. Broekaert, "cDNA cloning and molecular analysis of two self-incompatibility alleles from apple," *Plant Molecular Biology*, vol. 27, no. 3, pp. 499–511, 1995.

[5] P. De Franceschi, L. Dondini, and J. Sanzol, "Molecular bases and evolutionary dynamics of self-incompatibility in the Pyrinae (Rosaceae)," *Journal of Experimental Botany*, vol. 63, no. 11, pp. 4015–4032, 2012.

[6] R. S. G. Dreesen, B. T. M. Vanholme, K. Luyten et al., "Analysis of *Malus S*-RNase gene diversity based on a comparative study of old and modern apple cultivars and European wild apple," *Molecular Breeding*, vol. 26, no. 4, pp. 693–709, 2010.

[7] G. A. Janssens, I. J. Goderis, W. F. Broekaert, and W. Broothaerts, "A molecular method for *S*-allele identification in apple based on allele-specific PCR," *Theoretical and Applied Genetics*, vol. 91, no. 4, pp. 691–698, 1995.

[8] H. Sassa, T. Nishio, Y. Kowyama, H. Hirano, T. Koba, and H. Ikehashi, "Self-incompatibility (*S*) alleles of the Rosaceae encode members of a distinct class of the T_2/S ribonuclease superfamily," *Molecular and General Genetics*, vol. 250, no. 5, pp. 547–557, 1996.

[9] K. Kitahara and S. Matsumoto, "Sequence of the S10 cDNA from 'McIntosh' apple and a PCR-digestion identification method," *HortScience*, vol. 37, no. 1, pp. 187–190, 2002.

[10] S. Matsumoto, K. Okada, A. Kojima, K. Shiratake, and K. Abe, "S-RNase genotypes of apple (*Malus domestica* Borkh.) including new cultivars, lineages, and triploid progenies," *The Journal of Horticultural Science and Biotechnology*, vol. 86, no. 6, pp. 654–660, 2011.

[11] K. Kitahara, H. Fukui, J. Soejima, and S. Matsumoto, "Cloning and sequencing of a new *S*-gene 'Sg-RNase' (accession no. AB019184) from *Malus*×*domestica* Borkh. 'Indo' (PGR99-046)," *Plant Physiology*, vol. 119, p. 1567, 1999.

[12] J. Morita, K. Abe, and S. Matsumoto, "*S-RNase* genotypes of apple cultivars grown in Japan and development of a PCR-RFLP method to identify the S_6- and S_{21}-*RNase* alleles," *Journal of Horticultural Science & Biotechnology*, vol. 84, no. 1, pp. 29–34, 2009.

[13] K. Kitahara and S. Matsumoto, "Cloning of the S_{25} cDNA from 'McIntosh' apple and an S_{25}-allele identification method," *Journal of Horticultural Science and Biotechnology*, vol. 77, no. 6, pp. 724–728, 2002.

[14] S. Matsumoto, T. Eguchi, H. Bessho, and K. Abe, "Determination and confirmation of *S*-RNase genotypes of apple pollinators and cultivars," *Journal of Horticultural Science & Biotechnology*, vol. 82, no. 2, pp. 323–329, 2007.

[15] Apple Manual—ROP, Afgan Agriculture, 2008, http://afghanag.ucdavis.edu/a_horticulture/fruits-trees/apples/reports-apple/.

[16] Afghanistan Horticulture, Afghanistan Natural Horticulture Department Organization, http://afghanistanhorticulture.org/pages/Germplasm.aspx.

[17] M. R. Thomas, S. Matsumoto, P. Cain, and N. S. Scott, "Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification," *Theoretical and Applied Genetics*, vol. 86, no. 2-3, pp. 173–180, 1993.

[18] W. Broothaerts, G. A. Janssens, P. Proost, and W. F. Broekaert, "cDNA cloning and molecular analysis of two self-incompatibility alleles from apple," *Plant Molecular Biology*, vol. 27, no. 3, pp. 499–511, 1995.

[19] K. Kitahara, J. Soejima, H. Komatsu, H. Fukui, and S. Matsumoto, "Complete sequences of the *S*-genes, *Sd*- and *Sh*-RNase cDNA in apple," *HortScience*, vol. 35, pp. 712–715, 2000.

[20] S. Matsumoto, S. Komori, K. Kitahara, S. Imazu, and J. Soejima, "S-genotypes of 15 apple cultivars and self-compatibility of 'Megumi'," *Journal of the Japanese Society for Horticultural Science*, vol. 68, no. 2, pp. 236–241, 1999.

[21] S. Matsumoto, K. Kitahara, S. Komori, and J. Soejima, "A new *S*-allele in apple, 'Sg', and its similarity to the 'Sf' allele from 'Fuji'," *HortScience*, vol. 34, no. 4, pp. 708–710, 1999.

[22] S. Matsumoto and K. Kitahara, "Discovery of a new self-incompatibility allele in apple," *HortScience*, vol. 35, no. 7, pp. 1329–1332, 2000.

[23] S. Matsumoto, "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," *International Journal of Agronomy*, vol. 2014, Article ID 138271, 9 pages, 2014.