Review Article

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

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Breeding and cultivation of new apple cultivars are among the most attractive and important issues for apple researchers. As almost all apple cultivars exhibit gametophytic self-incompatibility (GSI), cross-pollination between genetically different cultivars and species is essential not only for stable fruit production, but also for breeding of new cultivars. For cross-pollination by insect or hand pollination, pollen viability and pistil fertility are key factors, but also the mechanism of GSI has to be taken into account. This paper reviews the germination rate of pollen after storage in different conditions, at different periods of flowering, and in combination with pistil fertility and cross-compatibility among wild-, crab-, and cultivated apples. Furthermore, suitable cultivar combinations for new attractive apple cultivars based on GSI are explored. Especially, details about S-genotypes of apple cultivars, which are present in recent cultivar catalogues, are introduced together with a newly established on-line searchable database of S-genotypes of cultivars, wild apples and crab apples that shows incompatibility, semicompatibility, and full- compatibility.

1. Introduction

Apple (Malus × domestica Borkh.) is one of the most important and widely cultivated fruit around the world. It is the second most important fruit (75.5 million t/year) in the world following bananas (107.1 million t/year) [1], and China is the largest producer, while the USA is second. Over 7,500 apple cultivars are known [2] and used not only for fresh consumption, but also for consumption as processed materials, such as juice, pie, or cider. Apple and apple products, including juices and extracts, have beneficial effects on Alzheimer’s disease, bone health, cognitive decline during normal aging, diabetes, and gastrointestinal protection from drug injury [3]. Apple is the most produced fruits in temperate climate areas and is expanding into subtropical and tropical areas [4]. However, recent global climate changes manifested in rising temperatures and late frosts in the flowering season caused poorer coloring of apple fruit skin and frost damage to pistils [5].

Pollination is a key event for stable apple production, and fertilization normally occurs between genetically different cultivars. In this review, I will focus on apple pollination, including pollen germination, pistil fertilization, and cross-compatibility. In addition, a searchable database system for apple cultivar combination evaluating the compatibility of cultivars is presented.

2. Pollen Storage and Germination and Pistil Fertilization

The time for flowering initiation is different among apple species and cultivars. For instance, pollenizers, including wild and crab apples, flower earlier than most other commercial cultivars. Although the pollen of early-flowering pollenizers cannot directly contribute to the pollination of commercial cultivars, it is desirable to store it as an additional pollen source for hand pollination. Actually, artificial pollination...
is necessary for stable apple production under unexpected weather conditions, such as low temperature not suitable for other pollinators. In some cases, stored pollen might be the used exclusively. Stored pollen is also important for breeding of new cultivars. For instance, the commercial cultivar “Orin” flowers a few days earlier than most other commercial cultivars. In this case, stored pollen of commercial cultivars has to be used for cross-pollination of “Orin” when aiming for new cultivars based on “Orin”.

Apple pollen keeps its viability for seven months if stored at −80°C under low relative humidity [6]. I investigated the germination rate of “Fuji” pollen, one of the most famous commercial cultivars according to the method described in Matsumoto et al. 2011 [7]. Results and discussion, as shown in Figure 1, are as follows. The viability of stored pollen was slightly reduced compared to fresh pollen but persisted up to six months at −20°C and −80°C. In contrast, viability was almost lost after six months at 4°C. The same tendency was also observed for “Maypole,” a typical pollenizer genotype (results not shown). Concluding from the results, it seems reasonable that stored pollen of pollenizers kept at 4°C should only be used as a pollen source for cultivars which flower during the same season. When pollen is stored with the intention of breeding or production in the coming season, storage should be performed at −20°C or −80°C.

Flowering period also differs among apple species and cultivars. The flowering period from the opening of the central flower to the abscission of the petals is influenced by the weather. It took eight days for “Gala”; nine days for “Elster,” “Golden Delicious,” “Granny Smith,” and “Fuji” at an average temperature of 15.8°C in 2007; 12 days for “Golden Delicious” and “Granny Smith,” 13 days for “Gala” and “Elster;” and 14 days for “Fuji” at 13.3°C in 2008 coupled with a lower amount of sunny hours at Nagylapos, Eastern Hungary [8]. In the case of commercial cultivars, the central flower opened first followed by the laterals [8]. I observed that the opening of the central flower of “Fuji” was between half a day and one day earlier than that of the laterals at Nagoya, Japan, in 2013 (the temperature during the flowering period at day time was 13–23°C). Also in the case of “Maypole,” the central flower opened first at all 5 to 7 flower clusters investigated. However, some clusters had two central flowers and opened one of these first (Figure 2(a)). The period between opening of the central flower and the opening of the lateral flowers was 1.5–2.5 days in “Maypole,” which was shorter compared to “Fuji” (3–4 days). The central flowers had an intense but short stigmatic activity, whereas lateral flowers had a discrete but much longer stigmatic activity [9]. Since many flower clusters of “Maypole” opened sequentially, the flowering period was longer (Figure 2(b)). The effective pollination period of “Golden Delicious” lasted for five to eight days [10]. In the case of “Fuji,” pistil fertility seemed to be maintained until four days after flowering at the same level regardless of pollination, but once fertilization occurred, the pistil lost its fertility within 24 hours [11]. Pollenizers in full bloom that are shifted more than 24 hours seem thus unnecessary at an orchard with only a single commercial cultivar, since repeated artificial pollination must be done within 24 hours [11].

3. Cross-Compatibility among Wild Apples, Crab Apples (Pollenizers), and Cultivars

As apple (Malus × domestica) shows gametophytic self- and cross-incompatibility, and at least two genetically distinct cultivars are necessary for stable apple production. Self-compatible apple cultivars are not known globally, but “Megumi” is one of the few self-compatible cultivars. However also here cross-pollination of “Megumi” is recommended for stable fruit production since the ratio of fruit set and seed number per fruit of Megumi × Megumi is 24.0–88.8% and 4.2, respectively [12].

The gametophytic self-incompatibility (GSI) system in apple is controlled by a multiallelic single locus, called S-locus [13, 14]. The genes responsible for apple GSI, (S-RNase as the pistil factor and SFB/SLF as the pollen factor) have been cloned [15, 16]. Both, S-RNase and SFB/SLF are located at the S-locus, and their combined alleles are called S-haplotype for they are tightly linked. In the GSI system, if one of the S-haplotypes of the pistil matches with that of pollen, pollen tube growth in the style is arrested. For instance, a cross between cultivars S1S2 and S1S2 results in cross-incompatibility, whereas a cross between cultivars S1S2 and S1S2 results in cross-compatibility. In the case of crossing S1S2 and S1S2, semicompatibility would occur since pollen of the S1-haplotype will be rejected by the S2-haplotype of the pistil, but that of S1-haplotype will not be rejected. Sometimes these semicompatible crossings cause undesirable fruit malformations, such as lopsided fruits. This might be caused by inadequate seeds in the fruit probably due to insufficient effective pollen at the time of pollination [11, 17, 18]. In addition to the S-haplotype, additional information about minor apple species and old cultivars, such as crab apples, is also important because apple orchards that consisted of a single cultivar, wild, and old cultivars have to be utilized as pollenizers for stable apple production. Moreover, information on the S-genotypes of apple is not only important.
for cultivar combination for stable apple fruit production, but also for the breeding of new cultivars.

Nowadays apple breeding using “Fuji” as either ovule or pollen parent is becoming increasingly popular in Japan and the genetic background of cultivars is becoming more homogenous. A lot of sports of “Fuji” were also found. For instance, cross-incompatibility occurs in any combinations within “Fuji” (S1S9), “Akifu 1” (S1S9; sport of “Fuji”), “Akifu 4” (S1S9; sport of “Fuji”), “Akifu 7” (S1S9S2S8), “Alps Otome” (S1S9), “Akiyo” (S1S9; “Senshu” × “Fuji”), “Aofu 1” (S1S9; sport of “Fuji”), “Aori 6 Gou” (S1S9S2S8; “Fuji” × “Jonathan”), “Benishigure” (S1S9; sport of “Fuji”), “Benishogun” (S1S9; sport of “Yataka”), “Fukunishiki” (S1S9; “Ralls Janet” × “Delicious”), “Gunfu 1” (S1S9; sport of “Fuji”), “Gunfu 2” (S1S9; sport of “Fuji”), “Hac 9” (S1S9; “Fuji” × “Tsugaru”), “Highland Fuji” (S1S9; sport of “Fuji”), “Hirosaki Fuji” (S1S9; sport of “Fuji”), “Hokuto” (S1S9S2S8; “Fuji” × “Rero11”), “Kaori” (S1S9; “Richared Delicious” × III Gou (“Ralls Janet” × “Delicious”), “Kinichifuhiro” (S1S9; sport of “Fuji”), “Korin” (S1S9; sport of “Fuji”), “Misora No Red” (S1S9; “Yoko” × “Senshu”), “Morifu 1” (S1S9; sport of “Fuji”), “Nagafu 1” (S1S9; sport of “Fuji”), “Nagara” (S1S9; sport of “Fuji”), “Nyoka No Kiseitsu” (S1S9; sport of “Fuji”), “Sairai” (S1S9), “Shinko” (S1S9; “Ralls Janet” × “Jonathan”), “Takano Wase” (S1S9; sport of “Fuji”), “Takita” (S1S9S2S8), and “Yataka” (S1S9; sport of “Fuji”). In addition, pollen viability of triploid cultivars, such as “Jonagold” and “Mutsu,” is low since triploids have 51 chromosomes and the chromosomes are unequally divided during meiosis. These cultivars cannot be used as pollinizers for other cultivars.

Using a S-RNase allele specific polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method developed by us [19, 20] and other groups in Belgium [21–24], China [25], Korea [26–28], Israel [29, 30], and U.S.A. [31], we have determined the S-RNase genotypes of new apple cultivars and selections produced in Japan to know their cross-compatibility with pollenizers and also to use them in a breeding program. Results and discussion are as follows. The S-RNase genotypes of K-13 and Kurosawa 4 Gou (Table 1 Nos. 2 and 9) failed to match any of the expected S-RNase genotypes based on their purported parents. We previously confirmed that “Komitsu” was produced by “Jonathan” × “Starking Delicious,” not by “Starking Delicious” × “Jonathan” [32]. Similarly, K-13 (S1S9; Table 1 No. 2) appeared to be derived from the cross “Shinano Gold” (S1S9) × “Tsugaru” (S1S9), rather than “Tsugaru” (S1S9) × “Shinano Gold” (S1S9). Another possibility of K-13 (S1S9) origin is a sport of “Tsugaru” (S1S9). “Kurosawa 4 Gou” (S1S9) also seemed to be a sport of “Fuji” (S1S9). From their fruit and branch characteristics, we speculated that the paternal parent and parents of K-15 (S1S9) and K-19 (S1S9) (Table 1, No. 4 and 8) were likely to be “Fuji” (S1S9) and “Shinano Gold” (S1S9) × “Tsugaru” (S1S9) or “Shinano Sweet” (S1S9). From the S-RNase genotypes of the remaining new cultivars and selections: Hayashi 7 Gou, K-14, K-16, K-17, K-18, Nakazawa 1 Gou, Redfield and RYOKU AP-10 (Table 1 Nos. 1, 3, 5–7, 10, 11, 14) matched one of the expected S-RNase genotypes from their reputed parents.

4. The Search System for Apple Cultivar Combination Showing Incompatibility, Semicompatibility, and Full-Compatibility Based on an S-RNase Allele Database

We have developed a search system for apple cultivar combination showing incompatibility, semicompatibility, and full-compatibility. Materials and methods are as follows. Construction of the search system began on the homepage using a JavaScript program. First, the apple S-RNase allele information program “apples-1.0.0.js,” which determines the operation on a homepage, was created using jQuery. Next, “index.html,” which converts picture information on a homepage to HTML format, was created. A file of three separate HTML forms was created as a framework in which search.
Table 1: S-RNase genotypes of 17 new apple cultivars including their selection.

<table>
<thead>
<tr>
<th>Number</th>
<th>Cultivar</th>
<th>Reputed parentage</th>
<th>S-RNase genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hayashi 7 Gou</td>
<td>Sport of &quot;Hayashi 1 Gou&quot; (S₁S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>2</td>
<td>K-13</td>
<td>“Tsugaru” (S₁S₇) × “Shinano Gold” (S₁S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>3</td>
<td>K-14</td>
<td>“Tsugaru” (S₁S₇) × “Sansa” (S₃S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>4</td>
<td>K-15</td>
<td>“Tsugaru” (S₁S₇) × Unknown</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>5</td>
<td>K-16</td>
<td>“Fuji” (S₆S₇) × “Goldroman” (S₄S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>6</td>
<td>K-17</td>
<td>“Fuji” (S₆S₇) × “Goldroman” (S₄S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>7</td>
<td>K-18</td>
<td>“Shinano Gold” (S₃S₇) × “Beniroman” (S₅S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>8</td>
<td>K-19</td>
<td>Unknown × Unknown</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>9</td>
<td>Kurosawa 4 Gou</td>
<td>“Fuji” (S₆S₇) × “Akane” (S₃S₂₄)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>10</td>
<td>Nakazawa 1 Gou</td>
<td>Sport of “Shinano Sweet” (S₃S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>11</td>
<td>Redfield</td>
<td>Wolf River (S₃S₉₆) × M. pumila Niedzwetzkyana (S₃S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>12</td>
<td>RYOKU AP-8</td>
<td>Chance seedling of “Shinano Red” (S₆S₉₀)</td>
<td>S₁S₁₀</td>
</tr>
<tr>
<td>13</td>
<td>RYOKU AP-9</td>
<td>Chance seedling of “Shinano Red” (S₆S₉₀)</td>
<td>S₁S₁₀</td>
</tr>
<tr>
<td>14</td>
<td>RYOKU AP-10</td>
<td>Sport of “Redfield” (S₃S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>15</td>
<td>RYOKU AP-11</td>
<td>Chance seedling of “Shinano Red” (S₆S₉₀)</td>
<td>S₁S₁₀</td>
</tr>
<tr>
<td>16</td>
<td>RYOKU AP-12</td>
<td>Chance seedling of “Shinano Sweet” (S₃S₇)</td>
<td>S₁S₇</td>
</tr>
<tr>
<td>17</td>
<td>RYOKU AP-13</td>
<td>Chance seedling of “Shinano Red” (S₆S₉₀)</td>
<td>S₁S₁₀</td>
</tr>
</tbody>
</table>

* Incorrect parentage indicated by this work is bolded.
+ Unknown: ovule or pollen parent is unknown.

Results are displayed on separate windows established for in-, semi- and full-compatibility. The search system homepage built in this manner can be accessed from the following URL: http://www.agr.nagoya-u.ac.jp/~hort/apple/ [7]. Information on the S-RNase allele nomenclature, the PCR-RFLP method including primers and PCR conditions, is also accessible via the search system homepage.

4.1. Database of Apple S-RNase Genotypes. Currently, we have identified the S-RNase genotypes of more than 600 apple cultivars and species using the S-RNase allele specific polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method [7]. The investigated S-genotypes included data of S-genotypes kindly provided by Dr. T. Akada and Dr. K. Sakurai. The names of the parent of each apple and additional reference information were compiled in a Microsoft Office Excel 12.3.2 file which was named “Apple-Sgene.xls” file. The created file was changed into a flat file “sample.xml” of XML format by using an Excel macro. The numerical number was input within the file for reference information, which was embedded on the retrieval program. Moreover, renewal of the XML file on a web server was performed using FTP soft FileZilla. Version up of the file through incorporating the published S-RNase allele data of cultivars in the world is under progressing [21, 23, 25–28, 30, 33–37].

4.2. The Search System for Apple Cultivar Combination Shows Full-, Semi-, and Incompatibility. The starting point of the search system is reached by accessing the URL (http://www.agr.nagoya-u.ac.jp/~hort/apple/) and entering an appropriate apple cultivar, species, or linage name in the box on the homepage. The search system will attempt to autocomplete the user entries. This way, even if the spelling of the name is ambiguous, the correct name can be easily chosen from the list of indicated candidates (Figure 3). Following the input of an appropriate name, the user has to decide whether the selected cultivar is used as either ovule parent (mother) or pollen parent (father). After clicking the search button, three windows show cultivars, species, or selections with in-, semi- and full-compatibility, respectively, displayed together with their S-genotypes and reference numbers (Figure 3). Full reference information is displayed as a pop-up by hovering the cursor over the reference number.

4.3. Data Handling among Diploids, Triploids, and Tetraploids Genotypes. Using the search system, combinations of apple cultivars, species, and selections showing in-, semi- and full-compatibility are easily obtained. For instance, if “Fuji” (S₆S₇) is chosen as an ovule or pollen cultivar, all of the in-, semi- and full-compatible apples with “Fuji” in the database, such as "Alps Otome" (S₁S₇), "Shinano Sweet" (S₃S₇) having one common S-RNase alleles of “Fuji,” "Shinano Sweet" (S₃S₇) having completely different S-RNase alleles with “Fuji”, respectively, are shown in each window.

In the case of diploid pollinizers which have novel unidentified S₆- and S₇-RNase alleles, cross-pollination can result in full-, semi-, or incompatibility (Figure 4(a)). In these cases, the search system displays all possible results in their respective windows.

Crosses of Diploid × tetraploid cultivars, triploid × tetraploid cultivars and tetraploid × tetraploid cultivars can result in full compatibility, incompatibility, or different degrees of semicompatibility, because diploid pollen can have more than one S-allele. For instance, pollinating S₆S₇ diploid
“Fuji” with \(S_1S_pS_q\) tetraploid “Tensei” pollen results in 50% semicompatibility, since only \(S_1S_p\) heteroallelic pollen, but not \(S_1S_q\) or \(S_pS_q\) homoallelic pollen, leads to fertilization in the pistil of “Fuji” (Figure 4(b)). Pollinating \(S_1S_q\) diploid “Jonathan” with “Tensei” pollen results in 75% semicompatibility, since only \(S_1S_q\) heteroallelic pollen, in addition to \(S_1S_p\) heteroallelic pollen, is not rejected in the pistil of “Fuji” [38].

Similarly, pollinating \(S_1S_2S_q\) triploid “Fukunishiki” with \(S_1S_pS_q\) tetraploid “Tensei” pollen results in 50% semicompatibility, but when \(S_2S_pS_q\) triploid “Jonagold” is pollinated with “Tensei” pollen 75% semicompatibility is observed (Figure 4(c)). Pollinating “Tensei” with \(S_1S_pS_q\) tetraploid “Welday Jonathan” pollen or “Welday Jonathan” with “Tensei” pollen results in 75% semicompatibility (Figure 4(d)). The search system does not distinguish between different degrees of semicompatibility and all semicompatible combinations are presented together in the corresponding window.

The results of triploid cultivar \(\times\) diploid cultivar and tetraploid cultivar \(\times\) diploid cultivar are displayed only on the basis of their S-genotypes. For instance, it is expected that triploid “Fukunishiki” \((S_1S_2S_p)\) \(\times\) diploid “Fuji” \((S_1S_q)\), “Fukunishiki” \(\times\) diploid “Shinano Sweet” \((S_1S_p)\), and “Fukunishiki” \(\times\) diploid “Tsugaru” \((S_1S_q)\) result in incompatible, semicompatible and full-compatible, respectively, and “Fukunishiki” \(\times\) diploid “Cox’s Pomona” \((S_1S_q)\), “Tensei” \(\times\) diploid “Snowdrift” \((S_2S_pS_q)\) and “Tensei” \(\times\) diploid “Dolgo” \((S_1S_q)\) result in semicompatible, full-compatible, and full-compatible, respectively (Figure 4(e)). Similarly, tetraploid “Tensei” \(\times\) diploid “Fuji” \((S_1S_p)\), “Tensei” \(\times\) diploid “Shinano Sweet” \((S_1S_p)\) and “Tensei” \(\times\) diploid “Tsugaru” \((S_1S_q)\) results in incompatible, semicompatible, and full-compatible, respectively, and “Tensei” \(\times\) diploid “Cox’s” “Pomona” \((S_1S_p)\), “Tensei” \(\times\) diploid “Snowdrift” \((S_2S_pS_q)\) and “Tensei” \(\times\) diploid “Dolgo” \((S_1S_q)\) result in semicompatible, full-compatible, and full-compatible, respectively (Figure 4(f)).

The determination of \(S-RNase\) genotypes of new cultivars and selections is an ongoing activity and new results will be implemented into the database through regular updates.

5. Suitable Cultivar Combination for Production of Novel Cultivars Including New Red-Fleshed Apple

Recently, the breeding of functional fruits containing biologically active components attracted attention. Anthocyanins are the main pigments in fruits. Anthocyanins possess strong antioxidant activity and are potent inhibitors of lipid peroxidation, which plays a role in the prevention of cardiovascular and neuronal illnesses, cancer, and diabetes [39]. In recent years, many studies have shown an effect of an anthocyanin-rich diet on the prevention of age-related neural disorders such as Alzheimer’s disease [40, 41].
In this chapter, I introduce a novel cultivar "Beniroman" and other new red-fleshed apples. "Beniroman" originated from a cross between "Shinano Red" and "Sansa" in 1998 at Kokaen in Iwate in northern Japan. Seeds were germinated in 1999, and 80 seedlings were budded onto JMI rootstock. Fruits were evaluated in 2008 and 2009, and "Beniroman" was selected. "Beniroman" came to full-bloom flowering two days before "Fuji" in "Kokaen" at 2008, which is typically at the end of April. "Beniroman" fruit ripens in the middle of August, which makes it one of the earliest cultivars in Japan. We made the cultivar in "Togokusan Fruit Park" located close to Nagoya in Aichi in central Japan, and surprisingly, it ripened at the end of July with high red pigmentation despite extremely high temperatures (33–35°C during daytime) (Figure 5). The S-RNase genotype of "Beniroman" was identified as S1S1. Development of novel cultivars derived from "Beniroman" with stable red skin pigmentation under high temperatures can be initiated using the search method as outlined in chapter 4.

There are two types of red-fleshed apples. Type 1 red-fleshed apples mainly were derived from Malus pumila var. Niedzwetzkyana and its derivatives show red pigmentation throughout the fruit; from fruit set through maturity [42]. In addition to the flesh and the skin of the fruits, vegetative tissues including leaves, branches, and floral organs are also highly pigmented, but in case of "Maypole," cortex pigmentation disappears gradually with maturity [42, 43]. Type 2 red-fleshed apples derived from "Pink Pearl" show a green-leaf and red-fleshed phenotype, and their cortex is specifically pigmented at the late stage of fruit development (Figure 5) [43]. We found that the red-fleshed trait of "Pink Pearl" is tightly linked with its S1-RNase allele located within the S-locus responsible for the gametophytic self- and cross-incompatibility system in apple [44]. Using this tight linkage, we found suitable cultivar combinations for efficient production of various red-fleshed apples (Table 2). Recently, new MYB-transcription factors named MdMYB110a and MdMYB110b JP responsible for red-fleshed trait in the type 2 were isolated from fruits from a cross between cv "Sciros" and a red-freshed cv "Sangrado" open pollinated (OP) seedling and JPP35 ("Jonathan" × "Pink Pearl"), respectively [43, 45].

6. Conclusion

Pollination is a key procedure during apple breeding and cultivation. In this paper, I introduced the mechanism of
apple self- and cross-incompatibility based on pollen-pistil interactions. Especially, I showed details about S-genotypes of apple cultivars which are present in recent cultivar catalogues. An online database (Search System) of S-genotypes of cultivars, wild apples, and crab apples was introduced that allows the user to search for apple cultivar combinations that result in incompatibility, semicompatibility and full-compatibility. This system will be of high value for anyone interested in apple breeding and cultivation.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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