Identification of Self- incompatibility Alleles in Some Almond Genotypes by Degenerate S-RNase Primers

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Abstract

The almond, Prunus dulcis Miller which belongs to Rosaceae family, is one of the most important commercial and oldest cultivated tree nut crops. Almonds are classified as a ‘nut’ in which the edible seed is the commercial product. Therefore, pollination and fertilization are necessary in almond. The characteristic of cultivated almond to express gametophytic self- incompatibility discourages self-fertilization and favors cross pollination. Genetic control of pollen-pistil self-incompatibility is through a single gene (S) which exists in a series of alleles S₁ to Sₓ. Compatibility of pollen-pistil in almond is an important consideration in planning crosses in breeding program and in choosing pollinizers for orchard planting. Identification of self-(in)compatibility in almond carried out by molecular and controlled pollination methods. In this study, identification of S-alleles in 37 Iranian almond cultivars and genotypes was carried out by PCR method with using degenerate primers of EM-PC3consRDEMPC2cons FD, PaconsIFand EM-PC1consRD. In this way the size of S-alleles were estimated based on bands which amplified with second intron. The results confirmed self-incompatibility in cultivars and most genotypes. However, the Sf-like allele (in size) was observed in A9 and A36 genotypes. If these results are confirmed by sequencing the Sf allele, it will be first time to identify self-compatible genotype in Iranian almond genotypes.

Keywords: Almond, Incompatibility, PCR, S-alleles.

Introduction

Almond is one of the most important nut trees in the Prunus genus. It belongs to the Rosaceae family. Most almond cultivars and genotypes are self-incompatible and some are cross-incompatible (Socias I Company and Alonso, 2004). Pollination, fertilization and commercial production require compatible pollen (Socias I company, 1990). Selecting cross-compatible cultivars with high quality pollen is the most important practice in almond orchard establishment (Sharafi, 2014; Kester et al., 1994). This trait is controlled by a single locus with multiple alleles and is expressed within the styles of flowers as S-RNAs Glycoprotein (Barklay et al., 2006; Wiersma et al., 2001; Halasz et al., 2007). These glycoproteins are responsible for the inactivation of self-pollen tube growth in most species of Prunus, including almond (Socias I Company and Alonso, 2004; Alonso and Socias I Company, 2006), apricot (Hajilou et al., 2006), sweet cherry (Wunsch et al., 2004) and plum (Yamane et al., 1999 Sutherland et al., 2004; Tamura et al.,1999).

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In a gametophytic incompatibility system, two genotypes with similar S-alleles cannot fertilize each other, but the presence of different S-alleles in two genotypes will result in successful fertilization. Cross-incompatibility will take place when two similar S-alleles are presented in pollen and style (Yamane et al., 1999; Sonneveld et al., 2003; Alonso and Socias I Company, 2006). Therefore, identifying cross-compatible cultivars and genotypes with favorable traits will be very beneficial for growers and breeders. However, the identification of S-alleles is essential for almond breeding programs to maximize the efficiency of crosses (Alonso and Socias I Company, 2006). Recently, methods based on DNA techniques to identify S-alleles pattern have been incorporated into fruit breeding programs in order to accelerate and optimize the determination of the (in)compatibility situation and S-alleles of fruit trees cultivars and genotypes (Sanchez-Perez et al., 2004; Lopez et al., 2006). However, controlled field and laboratory pollination are needed to confirm the effects of pollens on fruit quality and for selecting suitable pollinizers of cross-compatible cultivars and genotypes identified by PCR based methods (Lopez et al., 2006). Identification of incompatibility alleles in almond was amplified using degenerate primers which were designed for different species of the Prunus genus. Different combinations of these primers for amplification of S-alleles in almonds (Sharafi et al., 2010 and 2012; Sutherland et al., 2004; Ortega et al., 2005, Mousavi et al., 2011), cherry (Sonneveld et al., 2001; Sutherland et al., 2004) and apricot (Halasz et al., 2005; Zhang et al., 2008) were introduced. Fallah et al. (2014), studied pollen-pistil compatibility relationships among cultivars Tuono, “Shokofeh”, “Sahand” and five improved genotypes obtained from a breeding program based on their S-alleles profiles by PCR amplification using degenerate primers included Pa ConsI-F, EM-PC1consRD, EM-PC2consFD, and EM-PC3consRD. Theoretically, the use of the different degenerate and regular primers developed by Ma and Oliveira (2001a) should allow the amplification of the $SI$ and $Sf$self-incompatibility alleles and the self-compatibility allele $Sf$ in the present progenies with just three primercombinations. Moreover, it should be mentioned that self-fertile cultivars are very important for establishment mono cultivar orchards to produce uniform nut production. The self (in) compatibility situation of Iranian almond cultivars and genotypes, especially those obtained from breeding programs, has been poorly studied. Therefore, the objective of this study was the assessment of self (in) compatibility alleles in 37 Iranian almond cultivars and genotypes obtained from different regions, based PCR amplification.

Materials and Methods

Plant material and Genomic DNA extraction

The plant materials included two middle bloom almond cultivars including Shahroudi$_{21}$ and Shahroudi$_{18}$ as control and 37 Iranian genotypes (obtained from hybridization of TouUno ♂ cultivar by 230♀ genotype) planted in Shaded university collection. Genomic DNA was extracted using the procedure described by Doyle and Doyle (1987). The quantity of the DNA samples was determined using 2% agarose gel electrophoresis (Table 1).

<table>
<thead>
<tr>
<th>Autoclave</th>
<th>Chemical substance</th>
<th>Final concentration</th>
<th>Stock contraction</th>
<th>For 100cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Trise- HCl pH 8.0</td>
<td>100mM</td>
<td>2M</td>
<td>5cc</td>
</tr>
<tr>
<td>Yes</td>
<td>EDTA</td>
<td>20mM</td>
<td>0.5M</td>
<td>4cc</td>
</tr>
<tr>
<td>Yes</td>
<td>NaCl</td>
<td>1.4M</td>
<td>5M</td>
<td>28cc</td>
</tr>
<tr>
<td>No</td>
<td>CTAB</td>
<td>---</td>
<td>---</td>
<td>1gr</td>
</tr>
<tr>
<td>No</td>
<td>PVP-40</td>
<td>---</td>
<td>---</td>
<td>2gr</td>
</tr>
<tr>
<td>No</td>
<td>B- mercaptoethanol</td>
<td>---</td>
<td>---</td>
<td>2cc</td>
</tr>
</tbody>
</table>

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PCR Primers

A set of four specific degenerate primers were used to amplify S-alleles in the studied cultivars and genotypes. Degenerate primers Pa ConsI-F, EM-PC1consRD, EM-PC2consFD, and EM-PC3consRD were used (Table 2). For amplification, the second intron of the forward primers EM-PC2consFD and reverse primer EM-PC3consR (Sutherland et al., 2004) and amplification of the first intron of the forward primers PaConsI-F (Sonneveld et al., 2003) and reverse primer EM-PC1consRD (Ortega et al., 2005) were used.

Table 2. Primers information.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Forward (F)/ Reverse (R)</th>
<th>Intron</th>
<th>Sequence</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaConsI-F</td>
<td>F</td>
<td>First</td>
<td>5' (C/A)CTTGTTCTGT(C/G)TTT(T/C)GCTTTCTTC 3'</td>
<td>57°C</td>
</tr>
<tr>
<td>EM-PC1consRD</td>
<td>R</td>
<td>First</td>
<td>5' GCCA(C/T)TGGTG(A/C)ACAAA(C/T)TGAA 3'</td>
<td>57 °C</td>
</tr>
<tr>
<td>EM-PC2consFD</td>
<td>F</td>
<td>Second</td>
<td>5' TCAC(A/C)AT(C/T)CATGGCCTAT 3'</td>
<td>58 °C</td>
</tr>
<tr>
<td>EM-PC3consRD</td>
<td>R</td>
<td>Second</td>
<td>5' A(A/T)(C/G)CC(A/G)TG(C/T)TTGTTCCATTTC 3'</td>
<td>58 °C</td>
</tr>
</tbody>
</table>

S-alleles amplification

Amplification reactions were carried out in 20 μL volumes containing: 1x PCR buffer (100mM TrisHCl, pH 8, 500 mM KCl), 0.8 µL MgCl₂, 2 µL dNTPs, 1.2 µL of each primer (forward and reverse), 0.2 U Taq polymerase and 3 µL of genomic DNA. The PCR reaction program consisted of two minutes at 94°C for denaturation, primary, in 34 cycles with 10 seconds, temperature 94°C, two minutes at 57°C and two minutes at 68°C, followed by a five minute extension at 72°C (Fallah et al., 2014). After PCR, the products were stored at 4°C (refrigerator) until electrophoresis was performed (Fallah et al., 2014).

Electrophoresis of PCR products

PCR products were separated in 2% agarose gel using 0.5 Tris-buffer-EDTA buffers and were stained with ethidium bromide. The gels were photographed using UV light with UVitec gel documentation. The molecular sizes of the PCR products were estimated based on 3 kbp DNA ladder plus (Fallah et al., 2014).

Results

Degenerate primers used in this study were able to identify self-incompatibility alleles (S₁, S₂, S₃, S₅, S₇, S₉, S₁₁, S₂₀, S₂₂, and S₂₄) which reported in Fig. 1 and Fig 2. Fragments size was in the range of 330-1300 bp in all studied genotypes. An unknown band was observed in some genotypes (Fig.1).
Table 3. Amplified S alleles in studied almond cultivars and genotypes

<table>
<thead>
<tr>
<th>Number</th>
<th>Genotype</th>
<th>Product size (bp)</th>
<th>Object S allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shahroudi 18 (B1)</td>
<td>900 and ?</td>
<td>S3, S4</td>
</tr>
<tr>
<td>2</td>
<td>Shahroudi 21 (B2)</td>
<td>1080 and 875</td>
<td>S15, S14</td>
</tr>
<tr>
<td>3</td>
<td>A1</td>
<td>400(^1) and 570</td>
<td>S11, S6</td>
</tr>
<tr>
<td>4</td>
<td>A2</td>
<td>200-350(^1,2) and 1300</td>
<td>S3, S12</td>
</tr>
<tr>
<td>5</td>
<td>A3</td>
<td>400(^1) and 450</td>
<td>S11, S2</td>
</tr>
<tr>
<td>6</td>
<td>A4</td>
<td>330(^1) and 800 (^2,3)</td>
<td>S5, S11</td>
</tr>
<tr>
<td>7</td>
<td>A5</td>
<td>400(^1) and 620</td>
<td>S11, S4</td>
</tr>
<tr>
<td>8</td>
<td>A6</td>
<td>380 ? and ?</td>
<td>S3, S1</td>
</tr>
<tr>
<td>9</td>
<td>A7</td>
<td>400(^1) and 500</td>
<td>S11, S21</td>
</tr>
<tr>
<td>10</td>
<td>A9</td>
<td>1200 and 570</td>
<td>S9, S6</td>
</tr>
<tr>
<td>11</td>
<td>A10</td>
<td>750 and 1250(^2)</td>
<td>S3, S1</td>
</tr>
<tr>
<td>12</td>
<td>A11</td>
<td>380? and ?</td>
<td>S4, S1</td>
</tr>
<tr>
<td>13</td>
<td>A12</td>
<td>980? and 800</td>
<td>S4, S2</td>
</tr>
<tr>
<td>14</td>
<td>A13</td>
<td>500 and 570</td>
<td>S21, S6</td>
</tr>
<tr>
<td>15</td>
<td>A14</td>
<td>400(^1) and 600</td>
<td>S11, S10</td>
</tr>
<tr>
<td>16</td>
<td>A15</td>
<td>—</td>
<td>-----</td>
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<tr>
<td>17</td>
<td>A16</td>
<td>400(^1) and 450</td>
<td>S11, S2</td>
</tr>
<tr>
<td>18</td>
<td>A17</td>
<td>620(^1) and 550(^2)</td>
<td>S4, S11</td>
</tr>
<tr>
<td>19</td>
<td>A18</td>
<td>? and 875</td>
<td>S7, S14</td>
</tr>
<tr>
<td>20</td>
<td>A19</td>
<td>400(^1) and 500</td>
<td>S11, S21</td>
</tr>
<tr>
<td>21</td>
<td>A20</td>
<td>? and 500</td>
<td>S6, S21</td>
</tr>
<tr>
<td>22</td>
<td>A21</td>
<td>2600(^1,2) and 500</td>
<td>S4, S21</td>
</tr>
<tr>
<td>23</td>
<td>A22</td>
<td>—</td>
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<tr>
<td>24</td>
<td>A23</td>
<td>330(^1) and 530(^2)</td>
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<tr>
<td>25</td>
<td>A24</td>
<td>450(^1) and 700(^3)</td>
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<td>26</td>
<td>A25</td>
<td>450(^1) and 530(^2)</td>
<td>S2, S11</td>
</tr>
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<td>A26</td>
<td>450(^1) and 600</td>
<td>S2, S10</td>
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<tr>
<td>28</td>
<td>A27</td>
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<td>A28</td>
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<tr>
<td>30</td>
<td>A29</td>
<td>500(^1) and 450</td>
<td>S21, S1</td>
</tr>
<tr>
<td>31</td>
<td>A30</td>
<td>1300 and 500</td>
<td>S12, S21</td>
</tr>
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<td>32</td>
<td>A31</td>
<td>450(^1) and 500</td>
<td>S2, S21</td>
</tr>
<tr>
<td>33</td>
<td>A32</td>
<td>500(^1) and 1050</td>
<td>S21, S14</td>
</tr>
<tr>
<td>34</td>
<td>A33</td>
<td>? and 500</td>
<td>S6, S21</td>
</tr>
<tr>
<td>35</td>
<td>A34</td>
<td>400(^1) and 600</td>
<td>S12, S10</td>
</tr>
<tr>
<td>36</td>
<td>A35</td>
<td>330(^1) and 400</td>
<td>S6, S11</td>
</tr>
<tr>
<td>37</td>
<td>A36</td>
<td>1200 and 380</td>
<td>S6, S11</td>
</tr>
</tbody>
</table>

Alleles amplified with first (1) and second (2) intron primers; A; not amplified S alleles and, ?; shows unknown S-alleles.

Comparison of the allele’s size with (Table 3) S-alleles in gene banks revealed that the most of the fragments were in the size range of S-alleles of the gene bank. The results showed that the second intron, which was amplified with primers (forward primer EM-pc2consFD and backward EM-pc3consR), identified cultivars and genotypes bands as being in the range 400bp to 1720 bp. Table 3 also shows that no bands were identified in four genotypes A15, A23, A27, and A30, but at least one band identified in other 33 genotypes.
Discussion

The fragments amplified by the four degenerate primers Pa ConsI-F, EM-PC1consRD, EM-PC2consFD, and EM-PC3consRD were in the size range of 330-1300 bp in all studied genotypes (Table 3). These four primer pairs could not amplify all S alleles in all studied genotypes. An unknown band was observed in some genotypes (Fig. 1). However, in this research the primer for a second intron identified all genotypes and corresponded to research (Mousavi et al., 2011 and Mousavi et al., 2011).

Furthermore, according to these results Alonso et al., (2006), studied S-genotype of some almond cultivars using four primer combinations of S3F/S3R2, S3F/ConR, ConF/S3R1 and ConF/S3R2 and amplified the S3 allele satisfactorily. However, their study demonstrated that in S1Sf genotypes, the intensification of the S1 piece usually covered Sf amplification. In addition, the amplification of the piece of the S3 allele in the S3Sf genotypes covered or silenced the intensification of the self-compatibility allele (Sf); two very close bands were sometimes seen since the molecular weights of the two fragments are very similar. Also these results show that the SfSf and S1Sf genotypes cannot be differentiated with the ConF/ConR primers. These two genotypes would not be expected to be found together in the present families, but in plants with those genotypes in which ‘Tuono’ is the mother plant, S1Sf and SfSf could appear if accidental self-pollination occurred prior to ‘Tuono’ emasculation.

The specific primer mixture SfF/SfR, on the basis of the Sf intron sequences, suitably detected the self-compatibility allele (Sf) in many of the seedlings analyzed (Channuntapipat et al., 2003). A band corresponding to a 490-bp size piece was amplified in the self-compatible genotypes. Thus, Sf-allele specific recognition was attained using the SfF/SfR combina-
tion; the present work describes a proficient way to specifically recognize the $S_3$ allele.

Rahemi et al (2010) studied the $S$-alleles were in 96 wild almonds and related Prunus species from 10 taxonomic groups using six sets of primers including: three degenerate primer pairs (PaConsIF(FAM)/EMPC1consRD, PaConsIF(FAM)/EMPC3consRD, EM-PC2consFD/EM-PC3consRD), one general primer pair AS1II/AmyC5R, one allele specific primer pair (CEBASf/AmyC5R), and one set of multiplex primers (AS1II/CEBASf/AmyC5R). Their results showed that the primers, including the allele specific (CEBASf/AmyC5R), did not amplify any self-compatibility allele ($S_f$) in the samples and that alleles $S_9$, $S_2$, $S_{13}$, and $S_{25}$ had the highest frequencies (12.26, 8.39, 7.74, and 7.74 percent respectively). This result was also reported by Sharafi et al. (2010) and Mousavi et al., (2014) It should be mentioned that primers and genotypes in this study was different in compared with same reported studies and differences in results is acceptable.

Conclusions

The results showed that 37 studied genotypes were clearly self-incompatible and only four genotypes were unrecognized. These 4 primer pairs could not amplify all $S$ alleles in all studied genotypes. According to many past studies it was demonstrated that the identification of $S$-alleles of new cultivars and genotypes, obtained from breeding programs, is very helpful for planning future breeding and orchard establishment programs, especially for speedy selecting of pollinizers. However our results, showed that as seen in many past studies the use of PCR for $S$-genotype detection in almond germplasm is slowly succeeding, but that more information on the behavior of the primers in different genotypes is required if efficiency is to increase. $S$-genotype determination by PCR in breeding progenies is most positive when the $S$-alleles of the parents are known and specific primers for them exist but presently this is not generally the case. However, a $S_f$-like allele (in size) was observed in $A_{36}$ and $A_{36}$ genotypes. If these results are confirmed in the future studies by sequencing $S_f$ allele, it will be the first identification of self-compatible genotype in Iranian almond genotypes.

Acknowledgments

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References


