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ORIGINAL ARTICLE

Almond Breeding to obtain new Self-compatible Genotypes by Molecular Method

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ABSTRACT
Almond tree has tolerance to drought and its fruit has high nutritional value. In almond production, self –incompatibility is one of the major problems and to solve this, many breeding programs have been conducted around the world. In this study we used a couple of primers for identification and determination of S-alleles in self-compatible and self-incompatible almond genotypes. Also in this research We studied some wild genotypes to evaluate their S-allels .The results showed , using allele specific primers SfF-SfR, Cebador 2-Cebador8 and ConF-ConR ,we obtained respectively 450bp,1200bpand 1200bp bands in self-compatible genotypes .wear as using of AS1II-AmyC5R primers were produced 1100,1200,800 and 600 bp bands for S_1, S_f/S_3, S_2 and S_5/S_{25} respectively. As well as using two primers (Cebador1-Cebador3) produced a band related to S1 allele and using S3F-S3R2 we could recognized between S_3 and S_f bands produced by As1II and AmyC5R pair primers. The results showed S2F and S2R primers produced bands specified for S_2 alleles too.
***Keyword**. Almond, Self–compatibility, Self-incompatibility, PCR method.*

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INTRODUCTION

Almond self- incompatibility is one of the main problem in the world and one of the important aims of almond breeding is to obtain new self – compatible genotypes. In plants such as almond which their commercial part is their seed, self- incompatibility means higher costs in order to obtain financial product [1,5]. Therefore, it is highly important to find self- compatible genotypes of almond in different areas of the world. In this research, almond self- incompatibility alleles were studied by molecular method in Kerman province, Iran. Almond self -incompatibility is gametophytic type and is controlled by a multi-allelic locus (*S- locus*).In style .this characteristic is expressed by producing specific glycoprotein ribonucleases known as S-RNase [3,7]. In fact, the glycoprotein is responsible for prevention of pollen tube in style [3,7,4].

MATERIALS AND METHODS

In this study, in order to determine different self- incompatible genotypes and different self- incompatible genotypes in almond, DNA was extracted. Then different alleles were identified using specific primers of various areas of S-allele in PCR-based methods. In this study, in addition to the mentioned genotypes, five local samples of appropriate grown commercial varieties in Kerman Province of Iran were also studied.
To study the quality and quantity of the extracted DNA, DNA quantification or electrophoresis method on agarose gel was used. If the samples included a good value of DNA, they were diluted regarding the quantification with the ratio of about 1 µL of the sample and 20 µL of sterile distilled water and then 10 µL of the resulted solution is used for PCR experiments [8,9].

PCR reactions

Taq DNA Polymerase 2X-preMix Master Mix of GeneON Company was used in PCR reactions. Various primers were used for PCR reactions, each one of which was especially able to produce specific alleles.

At the first stage of the conducted experiments, temperature was held at 94° C for 4 minutes for initial denaturation. There were 35 cycles including two first stages, DNA denaturation and primer connection respectively, and the third stage with the temperature of 72°C for amplification of DNA [1].At this stage, the connection stage varies with the change in the used primer. As a result, PCR experiments by using two pairsprimers, AS1II and AMYC5R, which indicated the huge number of the studied alleles. Thus, these two primers can be used at the beginning of the experiments and the results can be compared to the results of the other studies (2 & 4). In addition to the mentioned primers, other pairs of primers such as Cebador2 and Cebador8, S3F and S3R2, and ConF and ConRwere also used [2,6].

RESULTS AND DISCUSSION

Various bands were formed using PCR experiments, the results of which are as follow.

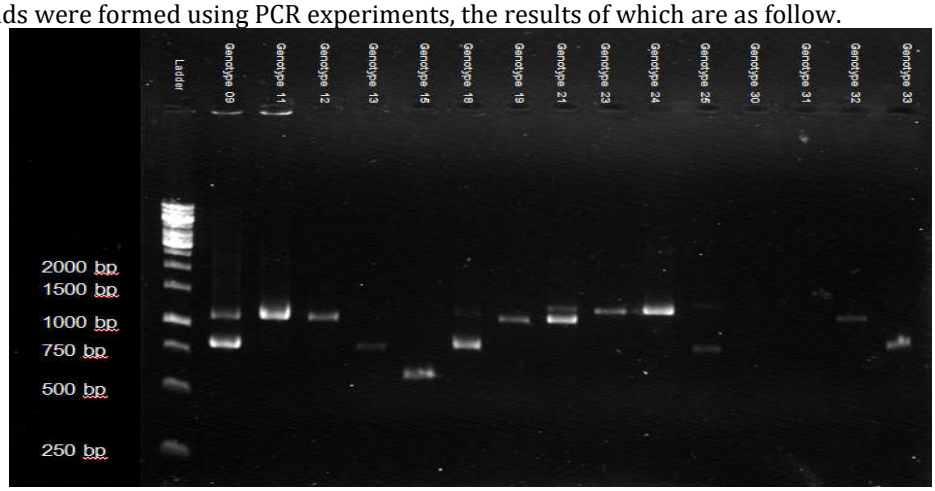

Fig1: The results of PCR reactions using a pair of primers, AS1II and AMYC5R.

The results of PCR reactions using a pair of primers, S3F and S3R2

Simultaneous use of a forwarding primer of S3F and a reveres primer of S3R2 resulted in amplification of S3- alleles in PCR reactions. The pair primers of S3F and S3R2 in S3- alleles confirmed the band with 790 Bp in genotype No. 24 and genotype No. 25 in which AS1II and AMYC5R have formed a band with 1200 Bp. In addition, it could amplify S3- allele in genotype No. 11 and form a band with 790 Bp.

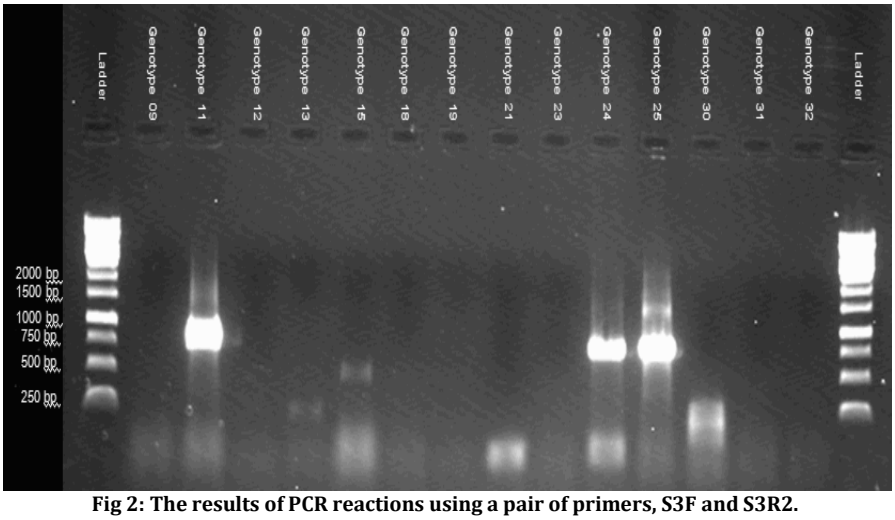

Fig 2: The results of PCR reactions using a pair of primers, S3F and S3R2.

As you can see in the figure 2, using this pair of primers resulted in forming a band with 449 Bp in genotype No. 21, the genotype which showed a band with 1200 Bp which was previously shown in this genotype belonged to self- compatible allele or Sf.

The results of PCR reactions using a pair of primers, Cebador2 and Cebador8

Sf- allele with a band with 1200 Bp in genotype No. 19 was produced using these primers which was not amplified while using other pairs of primers such as AS1II and AMYC5R as well as SfF and SfR (but as it will be mentioned, self- compatibility of this genotype will be confirmed since ConF and ConR primers

formed a band with 1200Bp for self-compatibility alleles). Therefore, the genotype No.19 has self-compatibility allele.

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