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Application of Random Amplified Microsatellite Polymorphism (RAMP) in *Prunus* Characterization and Mapping

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Abstract

Random amplified microsatellite polymorphism (RAMP) is a PCR-based marker which uses a combination of two classes of markers: Simple sequence repeat (SSR) and Random amplified DNA polymorphism (RAPD) markers. RAMP has been demonstrated to be a potentially valuable molecular marker for the study of genetic relationships in cultivated plant species. The objective of this study was to optimize the application of RAMP markers for the molecular characterization of a F_1 almond progeny from the cross between 'Tuono' and 'Shahrood-12' cultivar. In this first study, genomic DNA was extracted from young leaf tissues and PCR reactions were done using two nuclear SSR markers (assaying forward and reverse primers) and two selected RAPD primers. In addition, to check the transferability of these RAMP markers across *Prunus* genus, a F_1 apricot progeny from the cross between the North American cultivar 'Goldrich' and the Spanish 'Currot' was assayed. Results showed the dominant nature of these markers with a great abundance and transferability although with a reduced polymorphism.

Keywords: Almond, Fingerprinting, Molecular markers, *Prunus dulcis*, SSR.

Introduction

Simple sequence repeats (SSRs) markers are based on the PCR technique through the specific amplification of the conserved DNA sequence flanking repetitive DNA sequences (microsatellite loci) of the genome (Tauzt, 1989). These kind of molecular markers are becoming the markers of choice for fingerprinting studies for a wide range of plants. SSRs are abundant with high polymorphism, dispersed through the plant genome, and codominant inheritance (Gupta *et al*., 1996), but they are labor intensive and limited. On the other hand, random amplified polymorphic DNA (RAPD) markers are inexpensive but exhibit a low degree of polymorphism and reduced repeatability (Welsh and McClelland, 1990).

To compensate for the weakness of these two approaches (SSR and RAPD); a new marker called RAMP (Random amplified polymorphism microsatellite) was developed (Wu *et al*., 1994). RAMP markers involved a SSR primer which is used to amplify genomic DNA in the presence or absence of RAPD primers (Fig. 1). The resulting products can be resolved using submarine agarose electrophoresis. The amplification products derived from the anchored primer are only detected (Boopathi, 2013).

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Advantages of RAMP include high polymorphism, widely distributed throughout the genome, with an easily and low cost application. However, mixture interpretation is more difficult and repeatability could be reduced (Wu *et al*., 994; Provan *et al*., 1999). RAMP has been successfully used in diversity studies in different species of fruit trees including peach (Cheng *et al*., 2001) and pomegranate (Lihua *et al*., 2013).

The objective of this study was to optimize the application of RAMP markers for the molecular characterization of several almond and apricot progenies to evaluate their use for mapping.

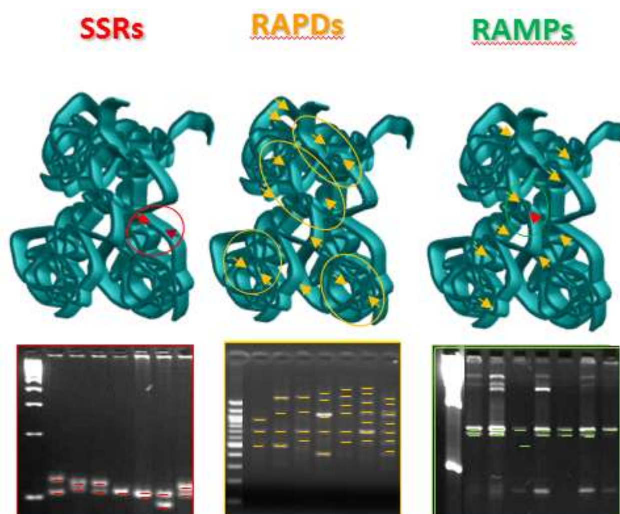

Fig. 1. Schematic representation of Simple sequence repeats (SSR), Random amplified polymorphic DNA (RAPD) and Random amplified polymorphism microsatellite (RAMP) markers

Materials and Methods

The plant material assayed included a F_1 almond progeny of seventy-eight seedlings from the cross between the Italian cultivar 'Tuono' and the Iranian cultivar 'Shahrood-12'. In addition, to check the transferability of these RAMP markers across *Prunus* genus, a F_1 apricot progeny from the cross between the North American cultivar 'Goldrich' and the Spanish cultivar 'Currot' was assayed.

Genomic DNA was extracted from leaves using CTAB method that was described by Doyle and Doyle (1987). In this first study, extracted DNA was PCR-amplified using a combination of two pair of primers (forward: TTGCTCAAAAGTGTCGTTGC and reverse ACACGTAGTGCAACACTGGC) flanking nuclear SSR sequences cloned in peach (UDP96003) (Cipriani *et al*. 1999) and apricot (UDAp473) (Testolin *et al.* 2004) and two selected RAPD primers in different almond genotypes (OPB11 and OPA8) (Rasouli *et al.* 2011). Amplification reactions were performed according to the protocol optimized by Sánchez-Pérez et al. (2006) to SSR markers assaying different annealing temperatures (from 35 ºC to 40 ºC) (Table 1). Amplified PCR products were separated using regular agarose (%3) and stained with GelRed™ (Biotium, Hatwad, CA, USA).

Table 1. Time and required temperature for PCR reactions by using RAMP primers.

Row	Steps	Temperature (˚C)	Time (min)	Number of Cycles
1	Initial denaturation	94	5′	1
2	Denaturation	94	1′	-
3	Annealing	35-40*	1′	35
4	Extension	72	2′	-
5	Final extension	72	10′	1
6	Hold	4	∞	1

*Depending on the type of primer is different.

Results

Annealing temperature is a critical step in optimizing the application of RAMP markers for the molecular characterization of almond (Fig. 2) and apricot (Fig. 3) genotypes. Annealing temperature of the anchored primers was usually 10-15 ºC higher than those of the RAPD primers. Thus, at a higher annealing temperature, only the anchored primers would anneal efficiently, whereas in PCR cycles at low annealing temperatures, both anchored SSR and RAPD primers would anneal.

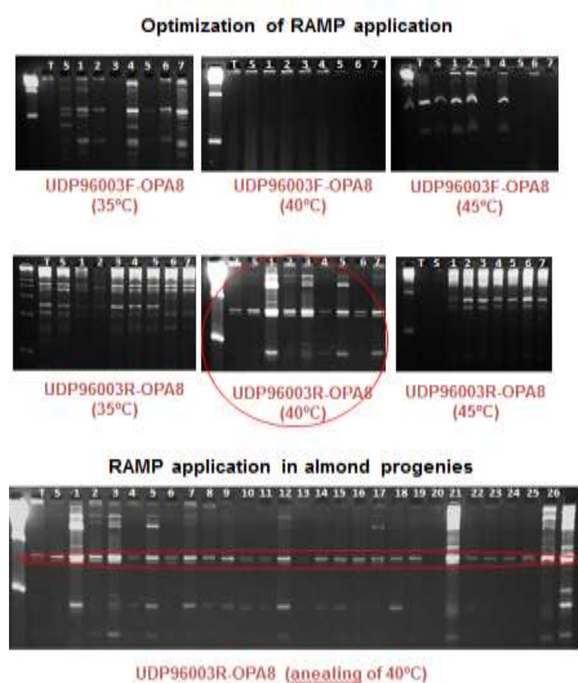

Fig. 2. RAMP optimization and application in the F_1 almond progeny 'Tuono' x 'Shahrood-12'using SSR primers [forward (F) and reverse (R)] from UDP96003 marker and RAPD primer OPA8, and assaying different annealing temperatures (from 35ºC to 45ºC).

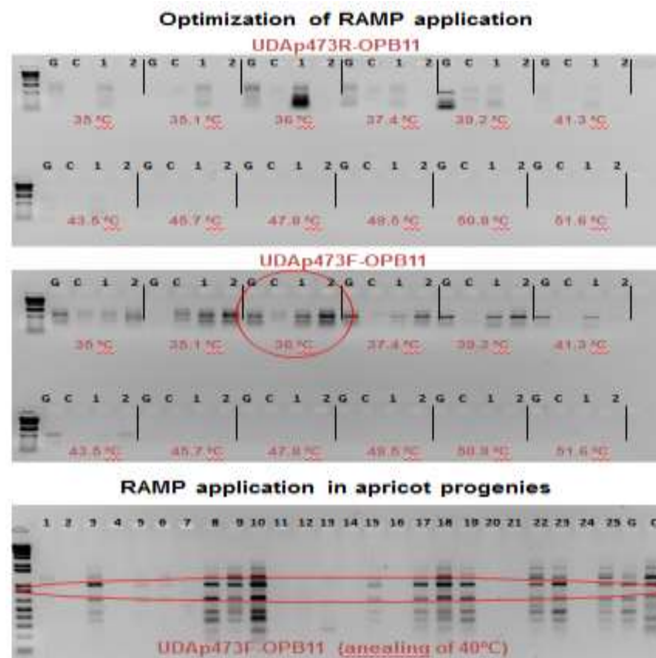

Fig. 3. RAMP optimization and application in the F_1 apricot progeny ‘Goldrich’ (G) x ‘Currot’ (C) using SSR primers [forward (F) and reverse (R)] from UDAp473 marker and RAPD primer OPB11, and assaying different annealing temperatures (from 35 ºC to 51.6 ºC).

The results showed the dominant nature of the RAMP markers, although with a reduced polymorphism. In addition, the great number of combinations of markers makes these markers very abundant. Finally, these results also confirm the transferability of these markers across the genus *Prunus*.

Discussion

Random amplified microsatellite polymorphism (RAMP; Wu *et al*., 1994) has been demonstrated to be another potentially valuable molecular marker for the study of genetic relationships in cultivated plant species. The combination of a simple sequence repeat (SSR; microsatellite) and a random sequence was used to amplify genomic DNA fragments in RAMP. RAMP has been employed in studies of the cultivars of barley (Wu et al., 1994; Becker and Heun, 1995; Sanchez de la Hoz *et al*., 1996). Molecular markers have been successfully used to map individual genetics factors or QTL controlling quantitative traits (Canli, 2004). The usual approach to analyze the association between marker–trait is a morphological and molecular study of populations segregating for particular agronomic characters of interest (Sánchez-Pérez *et al*., 2007). The analysis of co-segregation among markers and characters allow establishment of the map position for major genes and QTLs responsible for their expression (Sánchez-Pérez *et al*., 2007).

The usefulness of the RAMP molecular marker has not been widely tested in other plant species. The PCR program could be modified such that there is switching between high and low annealing temperatures during reaction. Most fragments obtained with RAMP primers alone disappear when a RAPD primer is included and when different patterns are obtained with the same RAMP primer and different RAPDs, indicating that the RAPD primer competes with RAMP primer during the low annealing temperature cycle.

Acknowledgments

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