Journal of Nuts 3(2):17-20, 2012
ISSN 2008-9937

# Optimization of the Analysis of Almond DNA Simple Sequence Repeats (SSRs) Through Submarine Electrophoresis Using Different Agaroses and Staining Protocols

*M.Rasouli [1*], A. Mousavi [2], B. Mohammadparast [3], P. Martínez-Gómez [4]*

[1] Department of Landscape Engineering, Faculty of Agriculture, Malayer University, Malayer, Iran
[2]Department of Horticulture, Agriculture and Natural Resources Research Center, Shahrekord, Iran
[3]Department of Biology, Faculty of Sciences, Malayer University, Malayer, Iran
[4]Department of Plant Breeding, CEBAS-CSIC, P.O. Box 164, 30100 Campus Universitario, Espinardo, Murcia, Spain

**Abstract:** Simple sequence repeat (SSR markers or microsatellites), based on the specific PCR amplification of DNA sequences, are becoming the markers of choice for molecular characterization of a wide range of plants because of their high polymorphism, abundance, and codominant inheritance. Different methods have been used for the analysis of the SSR amplified fragments being submarine agarose electrophoresis the more suitable method for the routine application. In this work we have performed a comparative study of the utilization of four different types of low melting (Metaphor®, Sea Kem®, and MS-8®) and regular (LD-2®) agaroses and two different staining protocols using Ethidium Bromide and Gel Red Nucleic Acid Gel Sating®. Almond cultivars assayed included the Spanish cultivars 'Antoñeta', 'Marta', 'Penta', 'Tardona' 'Desmayo' and 'Guara', the French cultivars 'Ferragnés' and 'R1000', the USA cultivar 'Mission', the Tunisian cultivar 'Achaak', the Italian cultivar 'Tuono' and the Australian cultivar 'Chellaston'. SSR detection using Metaphor® agarose gel electrophoresis was the most efficient with higher resolution and would be able to resolve most of allelic variation in comparison with the other three agaroses assayed. In addition, gel staining using Ethidium Bromide showed similar results than the GelRed™ Nucleic Acid Gel Stain® although it is much more toxic. The use of MetaPhor® agarose and GelRed™ Nucleic Acid Gel Stain® appears good indicated for molecular characterization of mapping of population due to its good resolution in comparison with the rest of agaroses, less toxicity in comparison with the use of Ethidium Bromide, and lower cost and easier routine application in comparison with the automatic capillary sequencing.

**Keywords:** Almond, *Prunus dulcis*, Breeding, Cultivar, Fingerprinting, Routine application, Molecular markers

## INTRODUCTION

DNA simple sequence repeats (SSR markers or microsatellites) also known as Short Tandem Repeats (STRs) are based on the PCR technique through the specific amplification of the conserved DNA sequence flanking repetitive DNA sequences (microsatellite loci) of the genome [8]. 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. They are well suited for the assessment of molecular genetic variability within crop species [2]. In addition, these DNA flanking SSRs are often well conserved in related species, which allows the cross-species

***Corresponding author:** M.Rasouli, Department of Landscape Engineering, Faculty of Agriculture, Malayer University, Malayer, Iran E-mail: m_rasouli83@yahoo.com

amplification with the same primer pairs in related species [3, 4].

Electrophoresis in polyacrilamide with radioactive and silver staining was the first method used in the analysis of the PCR amplified fragment of DNA obtained from the SSR markers. Metaphor® agarose was a method used as alternative to the polyacrilamide due to its easier application [5]. Low melting agaroses such as Metaphor® are derivated by organic synthesis which generates methoxylate groups from the basic agarose structure. The main properties of these agaroses are their low melting and gelling temperatures when compared with standard agaroses and the higher resolution in the analysis of DNA fragment. More recently, new methods for the PCR amplified DNA have been developed including the utilization of capillarity electrophoresis in automated sequencers. However, the use of submarine agarose gels continue being the easier method for the analysis of SSR markers although it appears less indicated for genotype characterization that the other two methods (and may be the most convenient in mapping of population [6, 7].

The objective of this work was the optimization of the analysis of almond PCR amplified DNA from simple sequence repeats (SSRs) through submarine electrophoresis using different low melting and regular agaroses and staining protocols.

## MATERIAL AND METHODS

Almond cultivars assayed included the Spanish cultivars 'Antoñeta', 'Marta', 'Penta', 'Tardona' 'Desmayo' and 'Guara', the French cultivars 'Ferragnés' and 'R1000', the USA cultivar 'Mission', the Tunisian cultivar 'Achaak', the Italian cultivar 'Tuno' and the Australian cultivar 'Chellaston'.

Total DNA was isolated using the procedure described by Doyle and Doyle (1987) and PCR-amplified using four pair of primers flanking nuclear SSR sequences cloned in peach (UDP96005, UDP98411, MA27a, MA40a)[1,3]. PCR reactions were performed according to the protocol optimized by Sánchez-Pérez et al., 2006 [6]. Amplified PCR products were separated using four different types of agaroses including low melting Metaphor® agarose (Cambrex, East Rutherford, NJ, USA) (with a prix around 2.50 €/g), low melting Sea Kem® agarose (FMC, Phyladelphia, NJ, USA) (1.53 €/g), low melting MS-8® agarose (Conda, Madrid, Spain) (1.72 €/g), and regular LD-2® agarose (Conda, Madrid, Spain) (0.85 €/g). In addition, two different staining protocols were also assayed including Ethidium bromide (MoBio, Carlsbad, CA, USA) (0.65 €/ml) and GelRed™ Nucleic Acid Gel Sating® (Biotium, Hatwad, CA, USA) (7.50 €/ml).

## RESULTS AND DISCUSSION

Results corroborated the suitability of the use of SSR markers for the assessment of molecular genetic variability in almond and the high degree of transportability between peach SSR in almond previously reported by Martínez-Gómez et al., 2003a and 2003b [3,4].

SSR detection using Metaphor® agarose gel electrophoresis was the most efficient and would be able to resolve most of allelic variation in comparison with the other agarose assayed (Fig 1). Regarding of the cost of the different low melting and regular agaroses assayed Metaphor® agarose is the most expensive (2.50 €/g) although the higher resolution quality justify its use.

On the other hand, gel staining using ethidium bromide showed similar results to than the GelRedTM Nucleic Acid Gel Sating® (Fig 2) although ethidium bromide is much more toxic. Regarding of the cost of the different staining protocols, GelRedTM Nucleic Acid Gel Sating® is around ten times more expensive although the safety in the lab and the lack of carcinogen risk justify its use.

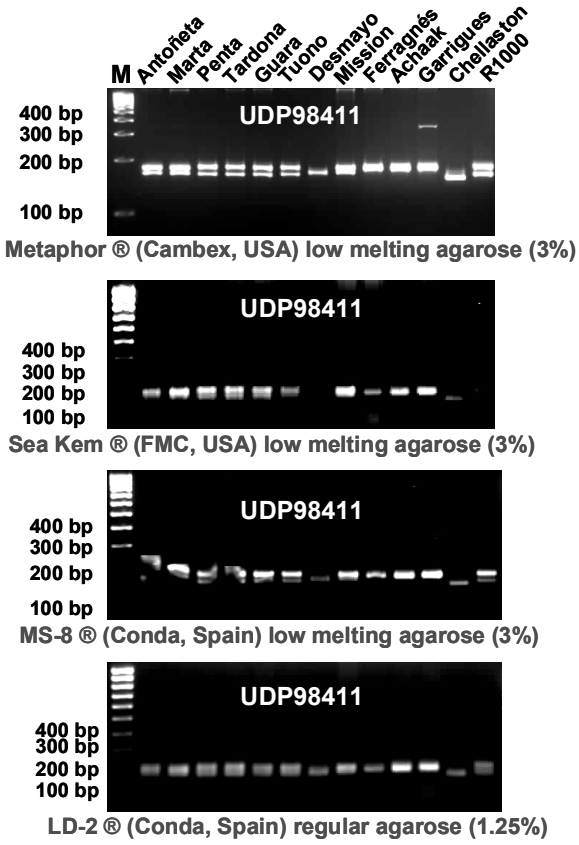


Fig 1. Metaphor®, Sea Kem®, MS-8®, and LD-2® agarose gels (3%) showing the allelic segregation of UDP98411 SSR marker assayed in 13 almond cultivars.

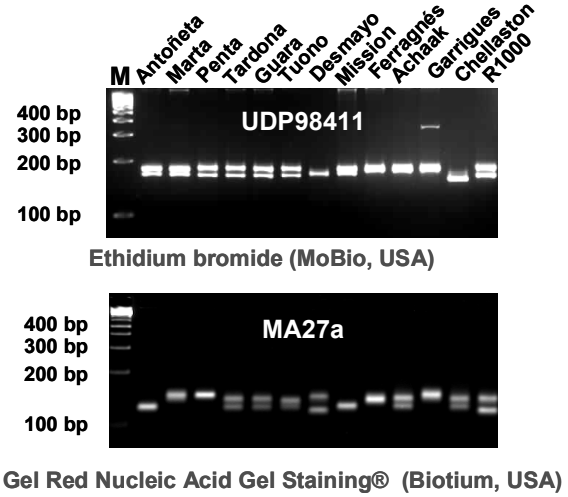


Fig 2. Metaphor® agarose gels (3%) showing the allelic segregation of UDP98411 and MA27a SSR markers assayed in 20 almond cultivars and stained using Ethidium bromide and GelRed™ Nucleic Acid Gel Sating®.

## CONCLUSION

As conclusion we can indicate that the use of MetaPhor® agarose and Gel Red Nucleic Acid Gel Sating® appears good indicated for molecular characterization of mapping of population, due to its good resolution in comparison with the rest of agaroses, less toxicity in comparison with the use of ethidium bromide, and lower cost and easier routine application in comparison with the automatic capillary sequencing.

## ACKNOWLEDGEMENTS

This work was financed by the project "Mejora Genética del Almendro", from the Spanish "Ministerio de Ciencia e Innovación". The assistance to this symposium was co-financed by Fundación Séneca of Región de Murcia, Spain.

## REFERENCES

1. Doyle, J.J. and Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.
2. Gupta, P.K., Balyan, H.S., Sharma, P.C. and Ramesh, B., 1996. Microsatellites in plants: a new class of molecular markers. Curr. Sci. 70:45–54.
3. Martínez-Gómez, P., Arulsekar, S., Potter, D. and Gradziel, T.M., 2003a. An extended inter-specific gene pool available to peach and almond breeding as characterized using simple sequence repeat (SSR) markers. Euphytica 131:313-322.
4. Martínez-Gómez, P., Arulsekar, S., Potter, D. and Gradziel, T.M., 2003b. Relationships among peach and almond and related species detected by SSR markers. J. Amer. Soc. Hort. Sci. 128:667-671.
5. Morgante, M., Pfeiffer, A., Jurman, I., Paglia, G. and Olivieri, A.M., 2003. PCR analysis of SSR polymorphisms in plant using agarose gels. In Karp A, Isaac PG, Ingram DS (eds) *Molecular tools for Screening* Biodiversity,

Kluwer Academic Publishers. The Netherlands. Pp 206-207.

6. Sánchez-Pérez, R., Ballester, J., Dicenta, F., Arús, P. and Martínez-Gómez, P., 2006. Comparison of SSR polymorphisms using automated capillary sequencers, and polyacrylamide and agarose gel electrophoresis: implications for the assessment of genetic diversity and relatedness in almond. Scientia Hort. 108:310-316.
7. Sánchez-Pérez, R., Howad, W., Dicenta, F., Arús, P. and Martínez-Gómez, P., 2007. Mapping major genes and quantitative trait loci controlling agronomic traits in almond. Plant Breed. 126:310-318
8. Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic DNA. Nucleic Acids Res. 17:6463–6471.