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in

Kodad O. (ed.), López-Francos A. (ed.), Rovira M. (ed.), Socias i Company R. (ed.).
XVI GREMPA Meeting on Almonds and Pistachios

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 119

2016
pages 111-114

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Abstract. Despite the new almond cultivars released from breeding programs are displacing the autochthonous cultivars in each area, there are a lot of local cultivars that still have a great interest and they are cultivated in the different areas. Some of them are difficult to identify and frequently the same name is given to different cultivars or a genotype is known with different names. These varieties were selected over the years for its adaptation to warm Mediterranean climate and they are a valuable plant genetic resource that must be protected. The objective of this work is the molecular characterization of Spanish autochthonous almond breeding collections using SSRs. Forty two different early flowering and self-incompatible cultivars were assayed using 5 SSRs of reference in almond: BPPCT007, CPPCT005, MA27a, MA40a and UDP96005. Results have showed the great diversity of this autochthonous germplasm and the effectiveness of SSRs by the correct identification, detecting some synonymies and suggesting the origin of some autochthonous cultivars.

Keywords. Almond – Germplasm – Characterization – Simple sequence repeat – SSR.

Caractérisation moléculaire des collections espagnoles autochtones d'amandier utilisant SSR

Résumé. *Bien que les nouveaux cultivars d´amandier issus des programmes d´amélioration remplacent les cultivars autochtones dans chaque région, il y a beaucoup de cultivars locaux qui ont encore un grand intérêt et ils sont cultivés dans les différents domaines. Certains d'entre eux sont difficiles à identifier et fréquemment, le même nom est donné à différents cultivars ou un génotype est connu avec des noms différents. Ces variétés ont été sélectionnées au fil des ans pour son adaptation au climat méditerranéen chaud et ils sont une ressource phytogénétique précieuse qui doit être protégée. L'objectif de ce travail est la caractérisation moléculaire des collections espagnoles autochtones d'amandier utilisant SSR. Quarante-deux différents cultivars à floraison précoce et auto-incompatibles ont été analysés en utilisant cinq SSR de référence pour l´amandier: BPPCT007, CPPCT005, MA27a, MA40a y UDP96005. Les résultats ont montré la grande diversité de ce matériel génétique autochtone et l'efficacité de la SSR dans l'identification correcte, la détection de certaines synonymies et pour suggérer l'origine de certains cultivars autochtones.*

Mots-clés. *Amandier –Germoplasme – Caractérisation – Simple sequence repeat – SSR.*

I – Introduction

Simple sequence repeats (SSR markers or microsatellites) characterized by their high polymorphism, abundance, and codominant inheritance, are becoming the markers of choice for fingerprinting studies for a wide range of plants including almond (Martínez-Gómez *et al*., 2003; Fernández i Martí *et al*., 2009). These markers are very useful in the development of DNA fingerprinting used in the identification of cultivars (Sánchez-Pérez *et al*., 2005). In this context the right characterization of autochthonous cultivars used in breeding programs is of especial interest. Some of these cultivars are difficult to identify and frequently the same name is given to different cultivars

or a genotype is known with different names. These varieties were selected over the years for its adaptation to warm Mediterranean climate and they are a valuable plant genetic resource that must be protected.

The objective of this work was the development of DNA fingerprinting using SSR markers for the molecular characterization of Spanish autochthonous almond cultivars from collections of CEBAS-CSIC of Murcia and IRTA of Constantí.

II – Materials and methods

Almond genotypes assayed included the 42 early flowering and self-incompatible local cultivars from the CEBAS-CSIC of Murcia and the IRTA of Constantí breeding programs (Table 1).

Total DNA was isolated using the procedure described by Doyle and Doyle (1987) and PCR-amplified using five pair of primers (BPPCT007, CPPCT005, MA27a, MA40a and UDP96005) flanking nuclear SSR sequences cloned in peach (Table 1). PCR reactions were performed according to the protocol optimized by Sánchez-Pérez *et al*., (2006). Amplified PCR products were analyzed by Automated Sequencer Capillary Electrophoresis using ABI PRISM® Genetic Analyzer (Applied Biosystems, Foster City, California, USA). The size standard used in the sequencer was Gene ScanTM 500 RoxTM using in the analysis the software GeneScan 3.7 (Applied Biosystems).

III – Results and discussion

Amplification of SSR loci was obtained for the five primer pairs developed in peach (BPPCT007, CPPCT005, MA27a, MA40a and UDP96005), all producing polymorphic amplification (Table 1). The number of alleles revealed by the SSR analysis ranged from 12 to 16 with a total number of polymorphic bands of 72. The mean number of alleles per locus was of 14.4 with heterozygosity between 0.80 and 0.89, and a discrimination power between 0.80 and 0.87.

The results leave no doubt that SSR markers are very suitable for identification of almond releases as indicated by different authors (Martínez-Gómez *et al*., 2003; Sánchez-Pérez *et al*., 2006; Fernández i Martí *et al*., 2009).

Results have showed the great diversity of this autochthonous germplasm and the effectiveness of SSRs by the correct identification, detecting some synonymies and suggesting the origin of some autochthonous cultivars. We should highlight the same fingerprinting found between ‘Desmayo AD’ and ‘Desmayo Lorca’, ‘Marcona AD’ and ‘Marcona’, ‘Planeta Fina’ and ‘Planeta Roja’, and between ‘Carreró’ and ‘Verd’, so they may be the same cultivars. Because of these markers present a great polymorphic nature, the fact of presenting the same profile is important evidence on these synonyms (Table 1).

On the other hand, these results showed the origin of the selection CEBAS-1 as a descendant of Garrigues and Carretas (Table 1).

Table 1. Spanish autochthonous almond cultivars assayed from the CEBAS-CSIC of Murcia and the IRTA of Constantí breeding programs and the SSR fingerprinting obtained with the analysis of five SSR markers

		SSR marker				
Cultivar	**Centre**	**BPPCT7**	**CPPCT5**	**MA27**	**MA40**	**UDP96-5**
Atascada	CEBAS	134	125/131	118/126	243/253	131/161
Atocha	CEBAS	134/160	125/151	128	195/253	156
Avellanera	CEBAS	134/150	131/133	126/128	219/253	161
Bonita	CEBAS	134/146	125	118/126	229/281	126/142
Carretas	CEBAS	134/142	125/142	107/153	248/277	156
CEBAS-1	CEBAS	142/146	125/146	126/153	221/277	126/156
Colorada	CEBAS	126/134	142/166	114/126	229/261	140/142
Del Cid	CEBAS	134/142	133/146	126/140	245/261	140/145
Desmayo AD	CEBAS	146/155	144/146	138/147	227/229	126/142
Desmayo Lorca	CEBAS	146/155	144/146	138/147	227/229	126/142
Fina del Alto	CEBAS	134/166	125/144	107/114	225/229	142/156
Fournat B.	CEBAS	160	133/159	138	221	126/156
Garrigues	CEBAS	134/146	144/146	126	221/255	126
J. Salazar	CEBAS	142/160	133	120/140	227/235	135/142
Jordi	CEBAS	142/166	144/157	130/138	221/225	140/156
La Mona	CEBAS	134	146/149	122/126	221/253	142/156
Malagueña	CEBAS	142/160	133/142	118/120	225/235	156/165
Marcona	CEBAS	134/162	133/149	120/126	263	142
Marcona AD	CEBAS	134/162	133/149	120/126	263	142
Marcona San Joy	CEBAS	134/153	149/151	116/140	221/253	175
Marcona Flota	CEBAS	134	146/149	120/126	227/261	142
Pajarera	CEBAS	144/162	125/151	118/126	225/261	142/175
Peraleja	CEBAS	142/150	133/144	112/118	221/225	140/159
Planeta Fina	CEBAS	134	146/162	118/126	225/253	126/142
Planeta Roja	CEBAS	134	146/162	118/126	225/253	126/142
Ramillete	CEBAS	134	146/162	118/140	213/225	126/142
Rumbeta	CEBAS	134	149/162	118/120	253/263	142/175
Tío Martín	CEBAS	158/172	133/151	118/147	221/225	126/156
Verruga	CEBAS	158	144/151	140/149	197/229	142/165
Angones	IRTA	142/146	131/144	114/138	221/227	126/142
Aspirilla	IRTA	146/153	144/146	120/138	227	129/142
Belardino	IRTA	134/155	133/142	126/138	225/263	142/156
Caima	IRTA	153/160	146	130	211	137/161
Carrero	IRTA	162/168	149/157	120/126	225/261	142/161
Esperanza Forta	IRTA	144	133/159	116/138	211/251	123/161
Gabaix	IRTA	153/158	146	134/138	207/223	137/161
Mollar de la Princesa	IRTA	160	133/159	138/140	221/225	126/161
Mollar de Tarragona	IRTA	155/160	131/144	116/138	211/245	156/161
Nano	IRTA	142/162	133/144	120/126	225/261	142
Parque Samá	IRTA	146/158	144/153	126/147	229	123/142
Pauet	IRTA	146/160	144	120/138	227	123/142
Pep de Juneda	IRTA	146/155	144/146	138/147	227/231	126/142
Rof	IRTA	153/162	144/146	130	211/213	156/161
Tardaneta	IRTA	153/160	144/146	116/130	211	137/161
Verd	IRTA	162/168	149/157	120/126	225/261	142/161

IV – Conclusions

Results showed the great diversity of this autochthonous germplasm and the effectiveness of SSRs by the correct identification, detecting some synonymies and suggesting the origin of some autochthonous cultivars.

Acknowledgements

This study was financed by the following projects: “Almond breeding” and “Prospection, characterization and conservation of endangered autochthonous almond varieties” by the Spanish Ministry of Economy and Competiveness.

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