Genetic diversity of a European collection of loquat (Eriobotrya japonica (Thumb.) Lindl.) determined by molecular markers

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Genetic diversity of a European collection of loquat [*Eriobotrya japonica* (Thumb.) Lindl.] determined by molecular markers

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SUMMARY – Loquat [*Eriobotrya japonica* (Thumb.) Lindl.] was introduced in Europe in the 18th century. It was first introduced as an ornamental tree. Later, when types with larger fruits were selected, it was grown because of its fruits. In Europe, it was grown in regular orchards at the beginning of the 20th century. At present, this species is an interesting alternative for the European fruit industry. A germplasm collection from a survey made in Spain and accessions introduced from Japan, Italy and Portugal was studied by means of RAPD markers. 34 highly reproducible markers were selected among 46 accessions. The polymorphism detected allowed us to distinguish 39 accessions. Our results suggested that, although a large number of new accessions were identified, the genetic diversity of loquat introduced in Europe is very low. It seems that a few forms were introduced from Japan. The species was propagated by seeds and developed many budsport, however the genetic base is narrow. To improve the identification of closely related accessions, eighteen sequences flanking microsatellites from *Malus domestica* L. were screened in a set of accessions. All sequences amplified SSR markers in the set of cultivars. According to these results, microsatellites are a useful alternative marker system for loquat genotyping.

Key words: *Eriobotrya japonica*, RAPD markers, SSR markers.

RESUME – *"Diversité génétique d'une collection européenne de néfliers [*Eriobotrya japonica *(Thumb.) Lindl.] déterminée par marqueurs moléculaires". Le néflier [*Eriobotrya japonica *(Thumb.) Lindl.] a été introduit en Europe au 18e siècle. Il a d'abord été introduit en tant qu'arbre ornemental. Ensuite, lorsque des types à plus grands fruits ont été sélectionnés, il a été cultivé pour ses fruits. En Europe, il a été cultivé dans des vergers réguliers au début du 20e siècle. A présent, cette espèce constitue une alternative intéressante pour l'industrie fruitière européenne. Une collection de germoplasme issue de prospections faites en Espagne et d'accessions provenant du Japon, de l'Italie et du Portugal, a été étudiée au moyen de marqueurs RAPD. Parmi 46 accessions, on a sélectionné 34 marqueurs fortement reproductibles. Le polymorphisme détecté a permis de distinguer 39 accessions. Nos résultats suggèrent que, bien qu'un grand nombre de nouvelles accessions ait été identifié, la diversité génétique du néflier introduit en Europe est très faible. Il semblerait que quelques formes aient été introduites à partir du Japon. L'espèce a été propagée par semences et a développé un grand nombre de mutations de bourgeons, mais cependant la base génétique est étroite. Pour améliorer l'identification d'accessions très proches, on a criblé dix-huit séquences de microsatellites flanquants de* Malus domestica *L. dans une série d'accessions. Toutes les séquences ont été amplifiées par marqueurs SSR dans la série de cultivars. Selon ces résultats, les microsatellites sont un système alternatif de marqueurs d'utilité pour le génotypage de néfliers.*

Mots-clés : Eriobotrya japonica*, marqueurs RAPD, marqueurs SSR.*

Introduction

Loquat [*Eriobotrya japonica* (Thumb.) Lindl.] was introduced in Europe in 18th century. First was introduced as ornamental tree. Later, when types with larger fruits were selected, it was grown because its fruits. In Europe, it was grown in regular orchards at the beginning of the 20th century. At present, this species is an interesting alternative into the European fruit industry. To improve loquat cultivation and preserve genetic resources, a collection was established in 1993 at the "Instituto Valenciano de Investigaciones Agrarias" (IVIA), Valencia, Spain. This collection included 81 accessions, 33 out of them were characterized by morphological characters (Badenes *et al.*, 2000) and RAPD markers (Vilanova *et al.*, 2001). The collection is being extended with new accessions yearly. Germplasm management and conservation of genetic resources could be accomplished if detailed characterization of plant material were available. The main problem encountered during the collection and evaluation of loquat species was the correct identification of accessions.

The development of molecular biology has significantly increased the pool of polymorphic markers for studies of crop diversity. DNA-based markers have several advantages over other marker types: (i) they are not affected by the environment; (ii) they can be detected in all tissues and stages of development; and (iii) using a combination of different approaches, many markers can be obtained. Among the PCR-based markers there are RAPD markers, random amplified polymorphic DNA (Williams *et al.*, 1990) and AFLP, amplified fragment length polymorphism (Vos *et al.*, 1995). Comparing both marker systems, RAPD technique needs simpler facilities and is less expensive. On the other hand, microsatellites or SSR (simple sequence repeats), short sequences containing tandemly repeated copies of 1-6 nucleotide fragments (Rafalski *et al*., 1996), have been shown as a good source of polymorphism very abundant in eukaryotic genomes. The aim of this study is to test the usefulness of RAPDs and SSR for genotyping loquat accessions closely related, as a tool for better germplasm management and future cultivar breeding.

Material and methods

The material used in this study, and its origin, is shown in Table 1. All cultivars used belong to the IVIA loquat germplasm collection. Genomic DNA was isolated from leaf samples following the CTAB (hexadecyltrimethylammonium bromide) method of Doyle and Doyle (1987). RAPD analysis was performed as described in Badenes *et al.* (1998). SSR analysis was performed as described by Romero *et al.* (2002). Sequences screened came from Guilford *et al*. (1997) and Gianfranceschi *et al*. (1998).

Table 1. Accessions studied and their origin. Those accessions that shared the same RAPDs profiles are followed by the same number (1) or (2)

Accession	Origin	Accession	Origin
1. Magdal Rojo Gordo	Spain	25. Pere Exquena.	Spain
2. Algerie	Spain	26. Cambrils	Spain
3. Ronda Brasil	Spain	27. Cort	Spain
4. Cox	Spain	28. Cabelo (2)	Spain
5. Saguntí	Spain	29. Silvia	Spain
6. Pallerés (1)	Spain	30. Polop-1	Spain
7. Temprano de Petrés	Spain	31. Redonet	Spain
8. Nadal Temprano	Spain	32. La Era	Spain
9. Manises.	Spain	33. Cayetano (1)	Spain
10. Alcacer-2	Spain	34. Faisca Redonda	Portugal
11. Manera	Spain	35. Poço Barreto	Portugal
12. Nadal Tardío	Spain	36. Mata Mouros	Portugal
13. Rosa Tardío	Italy	37. Almargem	Portugal
14. Ronda Gruesos (2)	Spain	38. Rolhâo	Portugal
15. Rosa	Italy	39. Tavira	Portugal
16. Marcheto	Italy	40. Masia la Cañera	Spain
17. Irma	Italy	41. Redonet-1	Spain
18. Vaniglia	Italy	42. Dama	Spain
19. Vaniglia Dulce	Italy	43. Siscar	Spain
20. Mas Vagué	Spain	44. Susana	Spain
21. Panisello 71	Spain	45. Cremaor (1)	Spain
22. Panisello 72	Spain	46. Barret (1)	Spain
23. Estrada Blanc	Spain	47. Flor de Invierno (1)	Spain
24. Estrada Groc	Spain		

Results and discussion

Among 46 accessions 34 markers highly reproducible were selected (Table 2). The polymorphism detected allowed to distinguish 39 accessions. Our results suggested that, although a large number of new accessions were identified, the genetic diversity of loquat introduced in Europe is very low. It seems that a few forms were introduced from Japan. The species was propagated by seeds and developed many budsport, however the genetic base is narrow.

Table 2. Polymorphic markers obtained in the set of cultivars studied. Markers are indicated by primer name and base pair size

Primers	Markers obtained
OPA-07	500, 650, 1050, 1100
OPA-08	650
OPA-09	900
OPA-11	550
OPB-07	600, 700, 800, 1200
OPC-09	950
OPJ-01	800, 1200
OPJ-05	600, 800, 1450
OPM-10	650, 800
OPP-06	1400
OPP-9	900
OPP-10	1400
OPP-16	625, 700, 800
OPY-08	1200
OPZ-05	1050
OPZ-06	1100, 450
OPZ-11	1500, 1200
OPZ-18	1000, 900

To improve the identification of closely related accessions, eighteen sequences flanking microsatellites from *Malus domestica* L. were screened in a set of accessions. All sequences amplified SSR markers in the set of cultivars (Table 3). According to these results, microsatellites are a useful alternative marker system for loquat genotyping.

Table 3. SSR from *Malus domestica* (Guilford *et al.,* 1997; Gianfranceschi *et al.,* 1998)

SSR name	Allele size range	Number of alleles	SSR name	Allele size range	Number of alleles
Col	213-239 bp	6	04h11	225 bp	6
02b1	238 bp	7	22c6	111 bp	3
05g8	121 bp	6	23f1	105 bp	2
28f4	112 bp	4	23g4	88 bp	9
CH02C06	216-254 bp	10	CH01B12	123-130 bp	5
CH01H10	93-119 bp	7	CH01E12	243-248 bp	8
01a6	136 bp	6	CH01F02	168-22 bp	11
01d7	117 bp	1	CH01F09	112-139 bp	9
01d12	126 bp	1	CH01H01	107-141 bp	9

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