Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2011 9(2), 453-459
ISSN: 1695-971-X
eISSN: 2171-9292

Simple sequence repeat (SSR) *vs.* sequence-related amplified polymorphism (SRAP) markers for *Cynara cardunculus* characterization

R. Casadevall[1], E. Martin[2] and V. Cravero[2]*

[1] *Facultad de Ciencias Agrarias (UNR). CC 14. S2125ZAA Zavalla (Santa Fe). Argentina*
[2] *CONICET. CC 14. S2125ZAA Zavalla (Santa Fe). Argentina*

Abstract

A little is known about the genetic variability present in globe artichoke, cultivated and wild cardoons. This knowledge is very important for efficient genetic resources utilization, and to gain a better understanding of genetic structure of this botanical varieties. With the aims to determine genetic distances between *Cynara cardunculus* accessions and to compare two molecular markers systems for their efficiency to differ between botanical varieties, a molecular characterization of sixteen accessions from different geographical origins was performed. Seven SSR and seven SRAP markers were used for varieties characterization and to calculate genetic distances between them. Both distance matrices were subjected to cluster analysis. Exclusive SSR alleles were found for globe artichoke and for wild cardoon, but non exclusive alleles were found for cultivated cardoon. For both markers systems two major groups were identified, one of them included mostly globe artichoke accessions and the other one grouped mainly cardoons. The differences observed in the sub-cluster conformation with each marker systems may be due to intrinsic characteristics of the markers. Concluding, both kind of molecular markers are valuable tools for studying genetic distances between *C. cardunculus* accessions although they give different information. Nevertheless, SSR electrophoretic profiles are simpler to score than SRAP markers because they consist of just a few bands. As well, bands are highly informative because of the great number of alleles existing in population and they are codominant markers. In addition, SSR's use would reduce time and costs.

Additional key words: cardoon; genetic resources; genetic variability; globe artichoke.

Resumen

Marcadores SSR (simple sequence repeat) *vs.* SRAP (sequence-related amplified polymorphism) en la caracterización de *Cynara cardunculus*

En la actualidad existe poca información acerca de la variabilidad genética presente en alcachofa y cardos (silvestres y cultivados). Conocer esta variabilidad es importante para utilizar eficientemente los recursos genéticos disponibles y comprender la estructura genética de estas variedades botánicas. Con el objetivo de determinar las distancias genéticas entre accesiones de *Cynara cardunculus* y comparar dos sistemas de marcadores moleculares en cuanto a su eficiencia para establecer diferencias entre las variedades, se realizó la caracterización molecular de 16 accesiones de diferentes orígenes geográficos. Para ello se utilizaron siete *primers* SSR y siete SRAP. Se calcularon las distancias genéticas y ambas matrices fueron sometidas a análisis de agrupamiento. Se encontraron alelos SSR exclusivos para alcachofa y cardo silvestre, pero no para cardo cultivado. El análisis de agrupamiento con ambos marcadores permitió identificar dos grandes grupos, el primero incluyó mayoritariamente accesiones de alcachofa mientras que el segundo agrupó a la mayoría de los cardos. Las diferencias observadas en la formación de sub-grupos con ambos sistemas se deberían a características intrínsecas de los mismos. En conclusión, ambos sistemas de marcadores resultan herramientas válidas para el estudio de distancias genéticas en *C. cardunculus* a pesar de que ambos brindan diferente tipo de información. Sin embargo, los patrones electroforéticos obtenidos con SSR son más simples de analizar que los SRAP ya que consisten en sólo unas pocas bandas, altamente informativas debido a que existe un elevado número de alelos codominantes en la población. Por otra parte, el uso de SSR permitiría reducir tiempos y costos.

Palabras clave adicionales: alcachofa; cardo; recursos genéticos; variabilidad genética.

* Corresponding author: vcravero@unr.edu.ar
Received: 10-05-10; Accepted: 23-02-11.

Abbreviations used: JDI (Jaccard's distance index), ORF (open reading frames), PCR (polymerase chain reaction), SRAP (sequence-related amplified polymorphism), SSR (simple sequence repeat).

Introduction

Cynara cardunculus L. is a diploid ($2n = 2x = 34$), perennial and cross-pollinated species belong to the *Asteraceae* family and native of the Mediterranean basin. The crop primary complex includes three botanical varieties: *Cynara cardunculus* var. *scolymus* (globe artichoke), *C. cardunculus* var. *cardunculus* (cultivated cardoon) and *C. cardunculus* var. *sylvestris* (wild cardoon) which are fully interfertile between them and their F_1 hybrids are fertile. The *Cynara* genus also includes other species: *C. syriaca* Boiss., *C. sibthorpiana* Boiss. et Heldr., *C. algarbiensis* Cosson, *C. baetica* (Spreng.) Pau (syn. *alba* Boiss.), *C. humilis* L., *C. cyrenaica* Maire & Weiller (Rottenberg and Zohary, 1996, 2005); *C. auranitiaca* and *C. tournefortii* (Robba *et al*., 2005). However, reproductive barriers separate the crop primary complex from the other wild *Cynara* species.

Globe artichoke heads or capitulum, which are immature composite inflorescences, are the edible parts of the plant and are used as a fresh, frozen or canned delicacy all over the world. Its cultivation is largely diffused in European Mediterranean countries and, to a lesser extent in South America, United States and, more recently, also in China (Bianco, 2005). The edible parts of cultivated cardoon are fleshy stems. It is cultivated in Spain, Italy and the south of France, where it is used in traditional dishes (Lanteri and Portis, 2007). In Argentina it is cultivated and consumed by European immigrant's descendants.

Alternative uses of these crops have been described in the last years, artichoke leaf extracts have been reported to inhibit cholesterol biosynthesis and to prevent cardiovascular disease. Also, artichoke and cardoon achenes are oil sources, and their biomass could be used for forage, paper pulp or biofuel (Maccarone *et al*., 1999; Gominho *et al*., 2000; Curt *et al.*, 2002; Quilho *et al*., 2004).

In contrast to other horticultural crops, no intensive breeding programs have been carried out for globe artichoke or cultivated cardoon (Sonnante *et al*., 2008). For this reason, a better knowledge of *C. cardunculus* genetics and variability is crucial in order to plan new breeding strategies, for efficient germplasm resources utilization and to maintain genetic diversity, which sustains long-term selection responses and reduces vulnerability (Sorrels and Wilson, 1997; Troyer *et al*., 1998; Jana, 1999).

Several authors analyzed the variability among *C. cardunculus* accessions using morphologic traits and biochemical markers (Dellacecca *et al*., 1976; Porceddu *et al*., 1976; Miccolis *et al*., 1989; Hammouda *et al*., 1993; Rottenberg *et al.*, 1996; Asprelli *et al.*, 2001). However, some of these markers are highly influenced by environmental conditions and give limited information, thus it is essential to include molecular characterization in diversity studies.

There are several kinds of molecular markers, each one brings different information and have its own advantages and disadvantages. Microsatellites, also known as simple sequence repeat (SSR) are tandem repeats of 1 to 6 bp which occur frequently in most eukaryotes genomes and can be highly informative since they are multiallelic and greatly reproducible. To amplify a particular SSR it is necessary to know the sequence flanking it to design usefull SSR primers. This can be laborious, time consuming and costly, then, the use of SSR technology depends usually, on the availability of suitable primers. Several SSR markers have been developed for *C. cardunculus* (Acquadro *et al*., 2003, 2005a,b, 2009), so it is not a limiting variable in this species.

On the other hand, sequence-related amplified polymorphism (SRAP) target open reading frames (ORFs). Primers consist of core sequences, which are 13 to 14 bases long, where the first 10 or 11 bases starting at the 5' end, with no specific constitution, followed by the CCGG sequence in the forward primer (to hybridize with exons which are CG rich regions) and AATT sequence in the reverse primer which target introns (AT rich). These core sequences are followed by three selective nucleotides at the 3' end which are variable from a primer to another (Li and Quiros, 2001). The advantages of this technology are that it is not necessary to know any sequence of genome under study and that this kind of markers is multilocus.

The aims of this study were to determine genetic distances between sixteen *C. cardunculus* accessions and to compare SSR with SRAP marker systems for their effectiveness in differing between globe artichoke and wild and cultivated cardoon.

Material and methods

Sixteen accessions of *Cynara cardunculus* L. including globe artichoke, wild and cultivated cardoon (Table 1), were sown in a completely randomized design in the Experimental Station of Rosario National University, located at Zavalla (33° 1' S; 60° 53' W), Argentine.

Table 1. List of *Cynara cardunculus* accessions geographical origins and botanical varieties

Accession	Geographical origin	Botanical varieties
1. Salanquet	France	scolymus
2. Violeta de Provenza	France	
3. Ñato	Argentine	
4. Romanesco	Italy	
5. Violetto Precocce	Italy	
6. Feltrin Roxa	Brazil	
7. Feltrin Verde	Brazil	
8. Gauchito FCA	Argentine	
9. Esmeralda FCA	Argentine	
10. 51A3	Argentine	
11. Farmer 1 (F1)	Argentine	cardunculus
12. Florensa S.A.	Argentine	
13. Semence S.A.	Argentine	
14. Farmer 2 (F2)	Argentine	
15. Farmer 3 (F3)	Argentine	
16. Route 9 (R9)	Argentine	sylvestris

Since this study did not aim to determine the presence of genetic variability within the accessions, five plants bulk of each accession were analyzed in order to represent the putative genetic variability present within accessions. Young fresh leaves of adult plants were ground to powder in liquid nitrogen and then genomic DNA was extracted using the PureLink Plant Total DNA Purification Kit (Invitrogen, Carlsbad, CA, USA). The resulting DNA was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Then it was quantified with commercial kit «QubitTM Flourometer (Invitrogen)» and stored at –20°C.

SSR protocols

Ten pairs of primers were tested (Table 2). SSR loci were amplified by the polymerase chain reaction (PCR) containing 50 ng DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM of each primer, 2 µL 10X Taq buffer and 1 U Taq polymerase (PB-L, Quilmes National University, Argentine) in a final volume of 20 µL. Samples were amplified by PCR with the following temperatures profile: 94°C for 5 min, 11 cycles at 94°C for 30 s, 60°C for 30 s decreasing by 0.5°C every cycle and 72°C for 1 min, finally 24 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 1 min (Acquadro *et al*., 2003, 2005a,b).

Table 2. Sequence of primers used for simple sequence repeat (SSR)

SSR	Primer pair sequence (5'-3')	Allele size range (bp)	Author
CMAFLP-04	F: CGATAGCTCTTTCCCTTT R: ATCCCTGAGATTGGTTTAC	310-360	Aquadro *et al.* (2005a)
CMAFLP-07	F: GGCTCCACCGTACTCCTA R: TCTCTCGTCAGTAAACCC	210-235	Aquadro *et al.* (2005a)
CMAL-02[1]	F: TAACCAAGAATATATCATGGAC R: AGAACCAGTTTTCATCAGAT	104-134	Aquadro *et al.* (2005b)
CMAL-6	F: CTGCAAGGAAAAGTAATCAT R: CCGATATAGATGAACCTAGC	140-164	Aquadro *et al.* (2005b)
CMAL-07[1]	F: AACCTGGTCGATGAATAATG R: GGTCTTGGTCTTCATGTCTC	160-184	Aquadro *et al.* (2005b)
CMAL-08	F: GAAGCAGATAATTGAAGTGTTG R: AATCCAATTCAACAAAATCTAC	150-174	Aquadro *et al.* (2005b)
CMAL-21	F: TAAATAGTTAGTGTTCTCGTTTG R TGGGGTTGTATTGGTTG	130-160	Aquadro *et al.* (2005b)
CMAL-24	F: GCCCGTTCACACAACA R: CAGGTTCTTTTTATACAGCAG	186-220	Aquadro *et al.* (2005b)
CMAL-25[1]	F: GAATCTAGTATACACAAGGTCA R: GGGTGCATAATGGTGATG	105-115	Aquadro *et al.* (2005b)
CMAL-108	F: TAACTAACCTCTAACATTGCCA R: AGAGAGCAATCTCTAACAAAG	86-92	Aquadro *et al.* (2005b)

[1] SSR that did not show robust amplifications profiles.

Amplification products were mixed with formamyde dye (98% formamyde, 10 mmol EDTA L^{-1}, 0.01% w/v bromophenol blue, and 0.01% w/v xylene cyanol), denatured at 95°C for 5 min and quenched on ice, separated on 6% (w/v) denaturing polyacrylamide gels, and visualized with silver staining (Bassam *et al*., 1991). Each SSR allele was scored as present (1) or absent (0).

SRAP protocols

Seven primers combination (Me1/Em4, Me1/Em5, Me2/Em4, Me3/Em2, Me3/Em5, Me4/Em3, Me4/Em4) were tested (Li and Quiros, 2001). Each PCR reaction sample was performed with 20 ng DNA, 5 mM dNTPs, 50 mM $MgCl_2$, 10 µM of each primer, 5.2 µL 10X Taq buffer and 4 U Taq polymerase (PB-L, Quilmes National University) in a final volume of 20 µL. Samples were amplified by PCR with the following thermal profile: 5 min at 94°C, 3 cycles of 1 min at 94°C, 1 min at 35°C and 1 min at 72°C, a 35 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C, with a final step of 1 min at 72°C. The amplification products were also visualized in denaturing polyacrylamide gels with silver staining. Bands were scored as present (1) or absent (0).

For each marker system, genetic distances were calculated according to standardized Jaccard's distance index (JDI) (Di Renzo *et al*., 2001). Both JDI matrices were subjected to cluster analysis and a dendrogram was created for each one using, in both cases, the Ward's method for InfoStat statistical software (Di Renzo *et al*., 2001). To test the dendrogram goodness of fit, the cophenetic correlation between the similarity matrix and the corresponding cophenetic matrix (computed from the tree matrix that generate the dendrogram) was calculated (Rohlf, 1974).

Results and discussion

Ten microsatellites were initially tested; three of them did not show robust amplification profiles and were discarded. Therefore, the analysis of the sixteen *Cynara cardunculus* accessions was performed with seven microsatellites, which gave one or two alleles per accession. The whole primers tested detect 25 alleles across the 16 accessions, ranging from 1 (CMAL-24) to 5 (CMAL-21 and CMAL-06) alleles per locus, with a 92% of polymorphism. Microsatellites are regions with high mutation rate, so such high percentages of polymorphism are very frequent (Matus and Hayes, 2002). Acquadro *et al.* (2005b) observed 65.9% of polymorphic alleles assessing genetic distances in a *C. cardunculus* population with SSR markers.

We found two SSR alleles exclusive for globe artichoke. One of them is a fragment of 140 bp obtained with the primers CMAL-21 and the other one is a fragment of 310 bp obtained with CMAFLP-04. Another two exclusive alleles were found for wild cardoon with the primers CMAL-21 and CMAFLP-07 (150 and 200 bp, respectively). No exclusive alleles were detected for cultivated cardoon. This fact may be a consequence of loose of genetic identity as a result of reiterating intercrossing between cultivated cardoon and the others botanical varieties (wild cardoon or globe artichoke). Acquadro *et al*. (2005b) by SSR markers amplified 6 distinctive alleles in globe artichoke and 26 in wild cardoon, but neither exclusive alleles were identified in cultivated cardoon, since each allele observed in cultivated cardoon was shared by at least one globe artichoke accession.

JDI obtained from SSR data ranged from 0.35 to 0.96 (Table 3). The lowest values were observed between both globe artichoke Brazilian accessions (Feltrin Roxa and Feltrin Verde) and between two Argentinian globe artichoke accessions (Gauchito and $51A_3$, both from the local breeding program). The highest JDI values were found between a wild cardoon accessions (R9) and two Argentinian globe artichoke varieties (Ñato and Gauchito). The average JDI was 0.73, indicating that the germplasm under study is fairly rich in diversity (Chen and Du, 2006).

Cluster analysis was able to identify two major groups. The cophenetic correlation was higher than 0.70 which indicates an acceptable goodness of fit of the similarity indices. One of the obtained groups (CI) included mostly globe artichoke accessions, whereas, the other one (CII) grouped mainly cardoons, with some exceptions (Fig. 1).

Cluster 1 (CI) was sub-divided into three sub-clusters. Each one was performed with accessions with common geographical origin, thus Feltrin Roxa and Feltrin Verde (from Brazil) were grouped together with the lowest JDI (0.35); Violetto Precocce and Romanesco, both Italian varieties, perform another sub-cluster (JDI = 0.63) together with a cultivated cardoon Semence (Argentine). Finally, Esmeralda, $51A_3$, Gauchito and Ñato, which are all Argentinean varieties obtained from the

Table 3. Jaccard's distances index matrix between the 16 accessions obtained by SRAP (above the diagonal) and SSR (below the diagonal)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	—	0.65	0.77	0.67	0.76	0.68	0.72	0.71	0.70	0.68	0.75	0.77	0.75	0.74	0.77	0.77
2	0.52	—	0.69	0.64	0.78	0.71	0.72	0.68	0.69	0.67	0.71	0.73	0.75	0.72	0.76	0.73
3	0.82	0.83	—	0.72	0.73	0.77	0.74	0.81	0.75	0.71	0.76	0.72	0.78	0.76	0.77	0.72
4	0.78	0.80	0.63	—	0.76	0.69	0.75	0.73	0.69	0.68	0.69	0.72	0.75	0.75	0.79	0.73
5	0.65	0.63	0.76	0.63	—	0.75	0.72	0.84	0.81	0.75	0.80	0.75	0.77	0.81	0.79	0.80
6	0.68	0.76	0.78	0.76	0.55	—	0.75	0.77	0.76	0.74	0.75	0.79	0.78	0.76	0.78	0.81
7	0.74	0.77	0.67	0.61	0.50	***0.35***	—	0.82	0.76	0.74	0.68	0.72	0.70	0.67	0.68	0.72
8	0.80	0.82	0.67	0.71	0.74	0.83	0.77	—	0.70	0.74	0.81	0.81	0.82	0.84	**0.85**	**0.85**
9	0.60	0.76	0.71	0.73	0.68	0.62	0.55	0.68	—	0.68	0.74	0.79	0.78	0.77	0.79	0.79
10	0.71	0.71	0.63	0.67	0.58	0.71	0.53	***0.35***	0.45	—	0.72	0.75	0.76	0.75	0.78	0.77
11	0.58	0.61	0.77	0.75	0.61	0.67	0.75	0.77	0.74	0.71	—	0.65	0.69	0.65	0.70	0.68
12	0.77	0.85	0.90	0.85	0.85	0.80	0.80	0.94	0.79	0.89	0.77	—	0.62	0.70	0.70	***0.58***
13	0.76	0.63	0.71	0.45	0.45	0.58	0.58	0.71	0.65	0.50	0.71	0.76	—	0.70	0.73	0.63
14	0.85	0.89	0.82	0.82	0.82	0.82	0.79	0.89	0.85	0.84	0.88	0.75	0.91	—	0.59	0.65
15	0.71	0.75	0.84	0.75	0.61	0.67	0.61	0.84	0.74	0.71	0.71	0.77	0.71	0.65	—	0.72
16	0.83	0.77	**0.96**	0.90	0.77	0.80	0.82	**0.96**	0.89	0.89	0.79	0.80	0.71	0.85	0.79	—

In italics, minimum JDI value. In bold, maximum JDI values.

local breeding program grouped together to perform the third sub-cluster.

On the other hand, the second cluster (CII) can subdivide into two sub-clusters. Most of the cultivated cardoons (F2, F3 and Florensa) grouped with the wild cardoon R9. The other sub-cluster was conformed to the other cultivated cardoon accession (F1) and the French globe artichoke accessions (Violetta de Provenza and Salanquet).

The analysis performed with SRAP data (270 polymorphic bands) showed an average JDI = 0.74

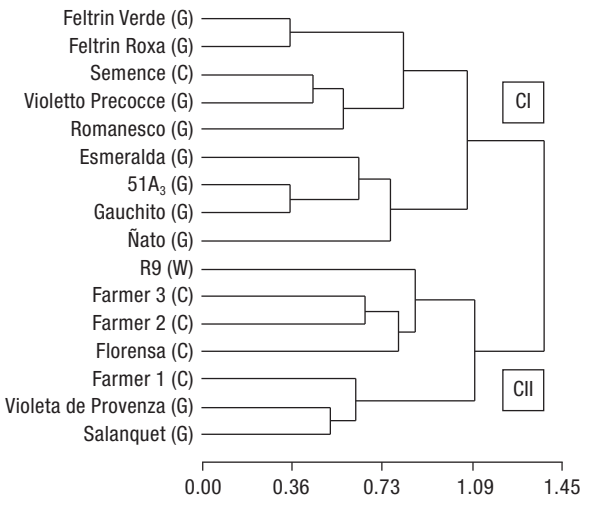

Figure 1. Dendrogram compiled by Ward method showing the grouping of the 16 *Cynara cardunculus* accessions based on SSR markers. G: globe artichoke. C: cultivated cardoon. W: wild cardoon. Cophenetic correlation coefficient = 0.72.

(Table 3) similar to that obtained with SSR data. Nevertheless, these JDI values presented a minor range of dispersion as it has been expected with data coming from coding sequences which tend to be more conserved. JDI's ranged between 0.58 (between cultivated cardoon Florensa and wild cardoon R9) and 0.85 (between Gauchito and F3 or Gauchito and R9). These results agree with those obtained with SSR in which Gauchito and R9 also show the largest distances from one to the other.

The dendrogram obtained with these JDI data also contained two main clusters (Fig. 2) in which cardoons and globe artichoke accessions appear separated one from the others (cophenetic correlation coefficient = 0.78). In this sense, Cluster 1 (CI) included all the cardoons accessions together with Feltrin Verde; whereas Cluster 2 (CII) included only globe artichokes, most of them grouped in an only one sub-cluster, except Ñato and Violetto Precocce which showed a JDI = 0.76 and conformed another sub-cluster.

Accessions F1, F2 and F3 together with Feltrin Verde perform a sub-cluster into the cluster 1, whereas the cultivate cardoons (Semence and Florence) and the wild cardoon accession grouped in another sub-cluster. Semence and Florence are both commercial varieties, whereas F1, F2 and F3 are three accessions collected in local producers' fields.

Both markers systems showed clusters with two main groups, one mostly conformed to globe artichoke accessions and the other one with cultivated cardoons,

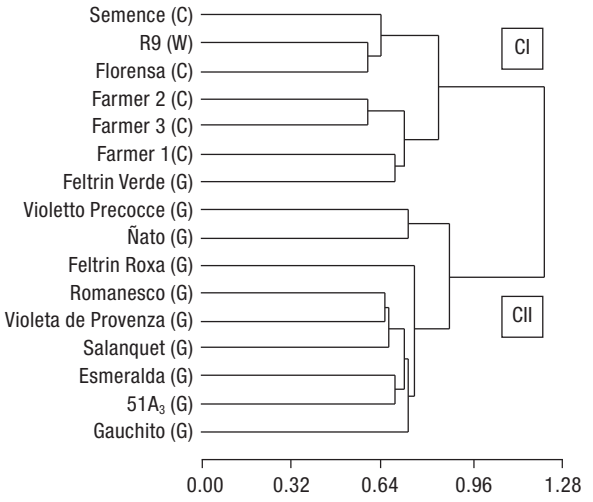

Figure 2. Dendrogram compiled by Ward method showing the grouping of the 16 *Cynara cardunculus* accessions based on SRAP markers. G: globe artichoke. C: cultivated cardoon. W: wild cardoon. Cophenetic correlation coefficient = 0.78.

nevertheless dendrograms were different in branching within each group.

The single wild cardoon included in the assay (R9) grouped with cultivated cardoons in both dendrograms. DNA studies (Lanteri *et al*., 2004; Acquadro *et al*., 2005a), cytogenetic- and isozyme-based analysis (Rottenberg *et al*., 1996) have all confirmed that wild cardoon is the ancestor of both cultivated *C. cardunculus* forms, which evolved independently under the influence of different anthropogenic selection criteria: globe artichoke for its capitula and cardoons for its succulent leaves. On the other hand, domestication of globe artichoke took place in the beginning of the first millennium while domestication of cardoon took place in the first half of the second millennium (Sonnante *et al*., 2007). Due to the low time since from both botanical varieties separation, it is reasonable that cultivated cardoons share more alleles with the wild progenitor than globe artichoke. In addition, both cardoon forms are full cross-compatible and seed propagated what may contribute to maintain a rather similar genetic background.

The differences observed in the sub-cluster conformation with each marker systems may be due to intrinsic characteristics of the markers. As Li and Quiros (2001) have pointed out, SRAP target ORFs, sequences which tend to be more conserved between genotypes than microsatellites which are generally non-coding regions, then, they are more variable. Clearly, the information given by both marker systems is different, which, results in not coincident dendrograms.

It is probable that if the number of SSR markers increase, the three botanical varieties identification will be improved. Acquadro *et al.* (2005b) genotyping 36 *C. cardunculus* accessions with 14 SSR, obtained three clusters, one of them contained only globe artichokes, other conformed to wild cardoons and the third one contained exclusively cultivated cardoon accessions.

SRAP technology give more bands per primer combination tested than SSR because of the random design of SRAP primers; possibly because of it, when we tried to obtain a dendrogram using the whole data acquired with both marker systems the tree was similar to the SRAP one.

Concluding, both kind of molecular markers are valuable tools for study genetic distances between globe artichokes and cardoons although they give different information. Nevertheless, SSR electrophoretic profiles are simpler to score than that resultant of SRAP markers because they consist of just a few bands. As well, bands are highly informative because of the great number of alleles existing in population and they are codominant markers. Also, SSR brings the possibility to perform multiplex-PCR increasing the information obtained in each experiment, reducing time and costs.

Acknowledgements

Financial support for this research work was provided by PICT 2007-00450 from the *Agencia Nacional de Investigaciones Científicas y Técnicas* (ANCYT), Argentina.

References

ACQUADRO A., LANTERI S., PORTIS E., 2003. Isolation of microsatellite loci in artichoke (*Cynara cardunculus* L. var. *scolymus*). Mol Ecol Notes 3, 37-39.

ACQUADRO A., PORTIS E., ALBERTINI E., LANTERI S., 2005a. M-AFLP-based protocol for microsatellite loci isolation in *Cynara cardunculus* L. (Asteraceae). Mol Ecol Notes 5, 272-274.

ACQUADRO A., PORTIS E., LEE D., DONINI P., LANTERI S., 2005b. Development and characterization of microsatellite markers in *Cynara cardunculus* L. Genome 48, 217-225.

ACQUADRO A., LANTERI S., SCAGLIONE D., ARENS P., VOSMAN B., PORTIS E., 2009. Genetic mapping and annotation of genomic microsatellites isolated from globe artichoke. Theor Appl Genet 118, 573-587.

ASPRELLI P.D., CRAVERO V.P., COINTRY E.L., 2001. Evaluación de la variabilidad presente en una población de clones de alcaucil (*Cynara scolymus* L.) Rev Inv Fac Cs Agr 1(1), 27-38. [In Spanish].

BASSAM B., CAETANO-ANOLLÉS G., GRESSHOFF P., 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196(1), 80-83.

BIANCO V.V., 2005. Present situation and future potential of artichoke in the Mediterranean Basin. Acta Hort 681, 39-55.

CHEN G., DU X.M., 2006. Genetic diversity of source germplasm of Upland cotton in China as determined by SSR marker analysis. Yi Chuan Xue Bao 33, 733-45.

CURT M.D., SÁNCHEZ G., FERNÁNDEZ J., 2002. The potential of *Cynara cardunculus* L. for seed oil production in a perennial cultivation system. Biomass Bioenerg 23(1), 33-46.

DELLACECCA V., MAGNIFICO V., MARZI V., PORCEDDU E., MUGNOZZA G.T., 1976. Contributo alla conoscenza delle varietà di carciofo coltivate nel mondo. Atti 2° Congresso Internazionale di Studi sul Carciofo. Turin, Italy. pp. 199-315. [In Italian].

DI RENZO J., BALZARINI M., CASANOVES F., GONZÁLEZ L., TABLADA M., GUZMÁN W., ROBLEDO C., 2001. InfoStat. Estadística y biometría. Versión 1.0. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina. [In Spanish].

GOMINHO J., FERNÁNDEZ J., PEREIRA H., 2000. *Cynara cardunculus* L.- a new fibre crop for pulp and paper production. Ind Crops Prod 13, 1-10.

HAMMOUDA R.M., SCIFEL-NASR M.M., ISMAIL S.I., SHAHAT A.A., 1993. Quantitative determination of the active constituents in Egyptian cultivated *Cynara scolymus*. Intl J Pharmacog 31, 299-304.

JANA S., 1999. Some recent issues on the conservation of crop genetic resources in developing countries. Genome 44, 562-569.

LANTERI S., PORTIS E., 2007. Handbook of plant breeding. Vegetables I. Springer, NY. Vol. 1, pp. 47-79.

LANTERI S., SABA E., CADINU M., MALLICA G.M., BAGHINO L., PORTIS E., 2004. Amplified fragment length polymorphism for genetic diversity assessment in globe artichoke. Theor Appl Genet 108, 1534-1544.

LI G., QUIROS C.F., 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103, 455-461.

MACCARONE E., FALLICO B., FANELLA F., MAUROMICALE G., RACCUIA S.A., FOTI S., 1999. Possible alternative utilization of *Cynara* spp. II. Chemical characterization of their grain oil. Ind Crop Prod 10(3), 229-237.

MATUS I., HAYES P., 2002. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 45, 1095-1106.

MICCOLIS V., BIANCO V.V., ELIA A., PERRINO P., VOLPE N., 1989. Valutazione della collezione mediterranea di carciofo allevata nella valle dell'Ofanto. L'Informatore Agrario 45, 35-41.

PORCEDDU E., DELLACECCA V., BIANCO V.V., 1976. Classificazione numerica di cultivar di carciofo. Proc II Intnl Congress on Artichoke. Bari, Italy. pp. 1105-1119. [In Italian].

QUILHO T., GOMINHO J., PEREIRA H., 2004. Anatomical characterisation and variability of the thistle *Cynara cardunculus* in view of pulping potencial. IAWA J 25(2), 217-230.

ROBBA L., CARINE M.A., RUSELL S.J., RAIMONDO F.M., 2005. The monophyly and evolution of *Cynara* L. (*Asteraceae*) sensu lato: evidence from the internal transcribed spacer region of nrDNA. Plant Syst Evol 253, 53-64.

ROHLF F.J., 1974. Methods of comparing classification. Ann Rev Ecol Syst 5, 101-113.

ROTTENBERG A., ZOHARY D., 1996. The wild ancestry of the cultivated artichoke. Genet Resour Crop Evol 43, 53-58.

ROTTENBERG A., ZOHARY D., 2005. Wild genetic resources of cultivated artichoke. Acta Hort 681, 307-313.

ROTTENBERG A., ZOHARY D., NEVO E., 1996. Isozyme relationships between cultivated artichoke and the wild relatives. Genet Resour Crop Evol 43, 59-62.

SONNANTE G., PIGNONE D., HAMMER K., 2007. The domestication of artichoke and cardoon: from roman times to the genomic age. Ann Bot 100(5), 1095-1100.

SONNANTE G., CARLUCCIO A., DEPAOLIS A., PIGNONE D., 2008. Identification of artichoke SSR markers: molecular variation and patterns of diversity in genetically cohesive taxa and wild allies. Genet Resour Crop Evol 55, 1029-1046.

SORRELS M.E., WILSON W.A., 1997. Direct classification and selection of superior alleles for crop improvement. Crop Sci 37, 691-697.

TROYER A.F., OPENSHAW S.J., KNITTLE K.H., 1998. Measurement of genetic diversity among popular commercial corn hybrids. Crop Sci 28, 481-485.