Genetic Diversity in *Cynara cardunculus* Determined by Sequence-related Amplified Polymorphism Markers

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ABSTRACT. Twenty-six wild and cultivated accessions of cardoon [*Cynara cardunculus* L. var. *sylvestris* (Lam.) Fiori and *C. cardunculus* var. *cardunculus* L., respectively] and globe artichoke [*C. cardunculus* var. *scolymus* (L.) Fiori] were analyzed to evaluate genetic diversity using sequence-related amplified polymorphism (SRAP) markers. Eleven qualitative and quantitative traits were evaluated and euclidean distances among genotypes were calculated. A total of 15 primer pairs were initially assayed and seven of them were finally selected as a result of their consistent amplification together with the clear banding patterns obtained. Genetic distances were calculated according to standardized Jaccard's distance index. Both matrices were subjected to cluster analysis. Dendrograms showed that cardoons were clearly separated from artichoke. These results showed that SRAP markers will be useful tools for studying genetic diversity in *C. cardunculus*.

Globe artichoke (*Cynara cardunculus* var. *scolymus*) originated in the Mediterranean basin, where another two botanical varieties are present: *C. cardunculus* var. *cardunculus* (the cultivated cardoon) and *C. cardunculus* var. *sylvestris* (the wild progenitor of globe artichoke). Artichoke propagation is mainly by vegetative means, but a high number of cultivars or ecotypes around the world (sometimes the same cultivar) have different names according to the region of cultivation (Sonnante et al., 2002).

An understanding of the magnitude and patterns of genetic diversity in crop plants has important implications for breeding programs and for conservation of genetic resources. Estimating the level of genetic diversity is of paramount importance for germplasm enhancement and for obtaining future genetic gain. Several authors analyzed the variability among cultivated and wild artichoke accessions using morphologic traits (Asprelli et al., 2001; Dellacecca et al., 1976; Elia and Miccolis, 1996; Miccolis et al., 1989; Porceddu et al., 1976). Comparative morphology suggested close relationships between globe artichoke and cardoon (Wiklund, 1992). More recently, cytogenetic test and isozyme comparisons (Rottemberg et al., 1996) confirmed this notion. Only a few molecular analyses have been conducted in this species. Lanteri et al. (2001), Sonnante et al. (2002), and Tivang et al. (1996) evaluated genetic diversity in artichoke using randomly amplified polymorphic DNA (RAPD) markers; Pagnotta et al. (2004) applied amplified fragments length polymorphisms (AFLPs) and inter-simple sequence repeats (ISSRs) to reveal variation within the 'Romanesco' population, and simple sequence repeat (SSR) markers

were recently developed in globe artichoke by Acquadro et al. (2003, 2005).

These kinds of markers could be used for germplasm evaluation. Each one has its own advantages and disadvantages. RAPD markers provide a simple polymerase chain reaction (PCR)-based molecular tool for the evaluation of genetic variation, but they have poor consistency and low reproducibility (Roodt et al., 2002; Welsh and McClelland, 1990). SSRs have the advantage of producing mostly codominant markers; however, their development is expensive and time-consuming (Li and Quiros, 2001). AFLPs are now widely used for genomic fingerprinting (Karaca et al., 2002; Zhang et al., 1999) as a result of their high multiplexing ratio (Vos et al., 1995). However, AFLPs are complex, require multiple steps, and have pseudopolymorphism when methylation-sensitive restriction enzymes are used (Li and Quiros, 2001).

Sequence-related amplified polymorphism (SRAP) technology has been recognized as a new and useful molecular marker system for mapping and gene tagging in *Brassica oleracea* L. (Li and Quiros, 2001), *Cucurbita moschata* (Duchesne ex Lam.) Duchesne ex Poir (Ferriol et al., 2004), and *Buchloe dactyloides* (Nutt.) Englem. (Budak et al., 2004). It targets coding sequences and can result in the identification of a number of codominant markers. SRAPs are based on two-primer amplification in which the primers are 17 or 18 nucleotides long. Primers consist of a core sequence of 13 or 14 bases in which the 5'-most 10 or 11 bases are nonspecific followed by the sequence CCGG in the forward primer and AATT in the reverse one. The core sequences are followed by three selective nucleotides at the 3' end of each primer (Li and Quiros, 2001). SRAPs are more reproducible than RAPDs and less complicated than AFLPs (Budak et al., 2004).

The objectives of this research were to determine if SRAP markers could be used to evaluate genetic diversity in a

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fragments was performed on 6% (w/v) polyacrylamide gels. Gels were 0.15 mm in thickness and 34 × 50 cm in dimension. Electrophoresis conditions were held at 75 W for 3 h 30 min at room temperature. The gels were stained with AgNO₃. SRAP fragments were visually scored as present (1) or absent (0).

Euclidean distances for the morphologic traits were calculated among genotypes. For SRAP analysis, genetic distances were calculated according to standardized Jaccard's distance index (JDI). Both matrices were subjected to cluster analysis, one dendrogram was created for morphologic traits and another was made for the molecular data using in both cases the Ward's method for InfoStat statistical software (Di Renzo et al., 2001). Bootstrap analysis was performed using the WinBoot program (Yap and Nelson, 1996) with 2000 repetitive sampling of the SRAP data to compute bootstrap *P* values.

Results and Discussion

MORPHOLOGIC CHARACTERIZATION. Cluster analysis for morphologic traits showed three main clusters or groups (Fig. 1). Discriminating values were obtained for all of the evaluated traits, except capitulum color. Those traits involved with capitulum size showed the highest discriminating values. Similar results in artichoke were found by Porceddu et al. (1976) who evaluated the variability for 27 characters and by Elia and Miccolis (1996) when they evaluated 104 accessions using 11 morphologic traits and obtained five main clusters.

In the present analysis, cluster 1 included all the genotypes of *C. cardunculus* var. *sylvestris* and all genotypes of *C. cardunculus* var. *cardunculus*. Genotypes included in this group have taller plants than the artichoke cultivars used in this experiment but no differences in plant diameter were observed (Table 2). These genotypes have a greater number of capitula but smaller size than the artichoke group. Only spiny forms were observed among wild cardoon accessions. In contrast, spiny together with spineless forms were found in cultivated genotypes. The presence/absence of spines appears to be a key to understanding the origin of the genotypes at present in

The PCR reaction mixture (20 μ L total volume) consisted of 20 ng genomic DNA, 5 mM dNTPs, 50 mM MgCl₂, 10 μ M of each primer, 5.2 μ L 10X Taq buffer, and four units of Taq polymerase. Samples were subjected to the following thermal profile: 5 min of denaturing at 94 °C and five cycles of three steps: 1 min of denaturing at 94 °C, 1 min of annealing at 35 °C, and 1 min of elongation at 72 °C; for the next 35 cycles, annealing temperature was elevated to 50 °C ending with a elongation step of 10 min at 72 °C. Separation of the amplified

Table 1. List of *Cynara cardunculus* cultivars analyzed in this assay and their origins.

<i>C. cardunculus</i> var. <i>scolymus</i>			<i>C. cardunculus</i> var. <i>cardunculus</i>		
	Cultivar	Origin		Cultivar	Origin
1	Salanquet	France	18	Horticulturist 1	Argentina
2	Caribou	France	19	Florensa S.A.	Argentina
3	Violeta de Provenza (entire leaves)	France	20	Semence S.A.	Argentina
4	Violeta de Provenza (indented leaves)	France	21	Horticulturist 2	Argentina
5	Ñato	Argentina	22	Horticulturist 3	Argentina
6	Imperial Star	U.S.			
7	Francés	Italy		<i>C. cardunculus</i> var. <i>sylvestris</i>	
8	Violetto Precocce	Italy	23	Buenos Aires	Argentina
9	Feltrin Roxa	Brazil	24	Colonia	Uruguay
10	Feltrin Verde	Brazil	25	Entre Ríos	Argentina
11	Gauchito FCA	Argentina	26	Santa Fe	Argentina
12	Gurí FCA	Argentina			
13	Estrella del Sur FCA	Argentina			
14	Oro Verde FCA	Argentina			
15	Esmeralda FCA	Argentina			
16	53B ₃	Argentina			
17	51A ₃	Argentina

Fig. 1. Dendrogram compiled by Ward's method showing the grouping of 26 *Cynara cardunculus* accessions based on morphologic traits.

cultivation; Barbieri (1959) hypothesized that the spiny types were selected first and then the violet types, which possess less spiny capitula, and finally the nonspiny types.

Globe artichoke accessions were distributed into clusters 2 and 3. Cluster 2 included mainly French genotypes, whereas cluster 3 contained materials obtained in the local breeding program in which plants were selected for high numbers of capitula and big size.

MOLECULAR CHARACTERIZATION. Fifteen primer combinations were initially tested for amplification of globe artichoke genomic DNA. Eight of them showed inconsistent amplification or low polymorphism and were discarded. Hence, the analysis of the 26 globe artichoke and cardoon accessions was performed with seven primer combinations. Pejic et al. (1998) reported that 150 polymorphic bands make it possible for a researcher to reliably estimate genetic similarities among genotypes within the same species; we found a total of 275 polymorphic fragments (an average of 39 polymorphic bands per primer combination) ranging in size from 80 to 1200 base pairs. Several authors (Budak et al., 2004; Ferriol et al., 2004;

Li and Quiros, 2001) have reported the presence of 10 to 20 polymorphic bands per primer combination. The higher polymorphism rate found in this study could be the result of the use of polyacrylamide gels with superior height and width (34 × 50 cm) giving them a higher resolving power.

The data obtained from SRAP analysis was used to calculate JDI. Distances ranged from 0.30 (for the two 'Violeta de Provenza' accessions 3 and 4) to 0.87 (between accessions 8 and 3). Sonnante et al. (2002) analyzed 37 wild and cultivated artichoke genotypes with RAPDs and obtained JDI ranging between 0 and 0.31. The highest distances values were obtained between *C. cardunculus* var. *sylvestris* accessions and cultivated globe

artichoke genotypes. It was noted that a value of 0 was only obtained between two spiny cultivars collected from different regions of Italy. The homogeneity that was observed between these two cultivars could be because they are a single cultivar, which was named differently according to the region where they are cultivated. Similar results were found for Lanteri et al. (2004) who analyzed 118 accessions, including 89 cultivar types, with AFLP. They report that the wild cardoon accessions analyzed clustered together and showed an average genetic differentiation of 64% from the *C. cardunculus* cultivated forms; the cultivated cardoon accessions were also differentiated from artichoke accessions but with lower JDI, whereas artichoke accessions were separated into two main clusters.

The cluster analysis performed with this molecular characterization detected two main groups with bootstrap *P* values above 75% (Fig. 2). One of them included only cultivated artichoke accessions (*P* = 81%) and the other comprised the wild and cultivated cardoons together with some artichoke cultivars (*P* = 68%).

Table 2. Mean values (MV) and standard deviation (SD) for the three clusters performed with all *Cynara cardunculus* genotypes considering morphologic data.

	Cluster 1		Cluster 2		Cluster 3	
	MV	SD	MV	SD	MV	SD
Plant ht (cm)	107.36	15.09	58.47	8.88	86.43	23.20
Plant diam (cm)	167.12	15.10	134.68	14.78	164.19	15.58
Capitula (no.)	10.63	1.07	4.64	0.74	6.16	3.44
Primary capitulum ht (cm)	4.84	0.92	9.57	1.07	9.20	0.66
Primary capitulum diam (cm)	4.96	0.90	8.51	0.79	9.61	0.49
Primary capitulum wt (g)	52.67	30.80	225.46	26.85	276.79	13.60
Secondary capitula avg ht (cm)	4.16	0.45	8.59	0.70	8.00	0.32
Secondary capitula avg diam (cm)	4.39	0.00	7.16	0.48	8.26	0.43
Secondary capitula avg wt (g)	38.96	0.95	135.25	1.37	176.46	0.39
Color (1–3 scale) ^z	1.20	0.66	1.68	0.80	1.43	0.46
Spine (1–2 scale) ^y	1.33	14.97	1.70	13.92	2.00	12.78

^z1 = green, 2 = purple-green, 3 = purple.

^y1 = spine presence, 2 = spine absence.

Fig. 2. Dendrogram compiled by Ward's method showing the grouping of 26 *Cynara cardunculus* accessions based on Jaccard's distances.

The tree node at JDI of 0.90 separates three artichoke cultivars from the cardoons ($P = 47\%$). Only 'Feltrin Verde' remained in the cardoon group. The lowest JDI was between the two 'Violeta de Provenza' accessions ($P = 99\%$). The close similarity was probably a result of a mutation in a single cultivar, which caused two distinct cultivars (one with entire leaves and the other with indented leaves). 'Violeta de Provenza' was propagated vegetatively, so simultaneous and indiscriminate propagation of the two types could have caused multiclonal composition of 'Violeta de Provenza' cultivars. The multiclonal composition in artichoke was also reported by De Vos (1986) for the American cultivar 'Green Globe' and by Tivang et al. (1996), who analyzed RAPD heterogeneity in two breeding populations of 'Green Globe' (genotypes not included in this study).

Morphologic traits produced better separations for artichokes than for cardoons. Although they have a common origin, they were exposed to different selection pressures. Although natural selection occurs in wild materials, human selection had a greater impact on cultivated varieties. Likewise, different objectives were proposed during domestication; cardoon was selected for its succulent leaves (Dellacecca, 1990), whereas artichoke was selected for high-quality capitula, possibly causing genetic divergence.

It must be noted that cardoon and artichoke genotypes are completely crosscompatible with fully fertile F_1 hybrids. Interspecific gene flow between closely related species may compromise the genetic integrity of botanical varieties. In consequence, the continuous molecular variation that was observed in this experiment could be the result of this fact. Acquadro et al. (2005) also reported continuous variation among *C. cardunculus* accessions. They found six exclusive SSR alleles in globe artichoke and 26 in wild cardoon, but no exclusive alleles were identified in cultivated cardoon. Indeed, globe artichoke and wild cardoon possess ribosomal genes of the same length (Maggini et al., 1988) that support the hypothesis that cardoon and globe artichoke have a common origin. Rottemberg and Zohary (2005) identify the wild forms of *C. cardunculus* as the wild progenitor stock of both cultivated forms.

However, despite this, cardoons and artichoke genotypes were positioned in different groups when SRAP markers were used. It is concluded from these results that SRAP could be a

helpful tool to detect *C. cardunculus* genetic diversity and to classify accessions into groups based on their distance values.

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